



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> C07K 14/40, C12N 1/00, 5/10, 15/31, 15/63, C12P 21/04, 21/06, C12Q 1/02, 1/68, G01N 33/53	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/36925</b> <b>(43) International Publication Date:</b> 9 October 1997 (09.10.97)
<b>(21) International Application Number:</b> PCT/US97/06170 <b>(22) International Filing Date:</b> 31 March 1997 (31.03.97)  <b>(30) Priority Data:</b> 626,309 1 April 1996 (01.04.96) US  <b>(71) Applicants:</b> SCRIPTGEN PHARMACEUTICALS, INC. [US/US]; 200 Boston Avenue, Medford, MA 02155 (US). PRESIDENT & FELLOWS OF HARVARD COLLEGE [US/US]; 124 Mt. Auburn Street, Cambridge, MA 02138 (US).  <b>(72) Inventors:</b> BURATOWSKI, Stephen; 706 Webster Street, Needham, MA 02912 (US). BURATOWSKI, Robin; 706 Webster Street, Needham, MA 02912 (US). WOBBE, C., Richard; 57 Spring Street, Lexington, MA 02173 (US). BRADLEY, John; 25 Parkman Street #1, Brookline, MA 02147 (US).  <b>(74) Agents:</b> LUDWIG, S., Peter et al.; Darby & Darby P.C., 805 Third Avenue, New York, NY 10022 (US).		<b>(81) Designated States:</b> CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> CANDIDA ALBICANS TATA-BINDING PROTEIN, NUCLEIC ACID AND ASSAYS  <b>(57) Abstract</b>  The invention encompasses a novel transcription factor from <i>Candida albicans</i> , TBP, a nucleic acid sequence encoding TBP, and methods of screening for inhibitors of <i>Candida albicans</i> growth by targeting TBP.		

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## CANDIDA ALBICANS TATA-BINDING PROTEIN, NUCLEIC ACID AND ASSAYS

ABSTRACT OF THE DISCLOSURE

15

The invention encompasses a novel transcription factor from *Candida albicans*, TBP, a nucleic acid sequence encoding TBP, and methods of screening for inhibitors of *Candida albicans* growth by targeting TBP.

The invention relates in general to transcription factors and to methods for screening for antifungal agents.

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The invention was made in part using government funds, NIH grant no. GM46498, and therefore the U.S. government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The yeast *Candida albicans* (*C. albicans*) is one of the most pervasive  
25 fungal pathogens in humans. It has the capacity to opportunistically infect a diverse spectrum of compromised hosts, and to invade many diverse tissues in the human body. It can in many instances evade antibiotic treatment and the immune system. Although *Candida albicans* is a member of the normal flora of the mucous membranes in the respiratory, gastrointestinal, and female genital tracts, in such locations, it may gain  
30 dominance and be associated with pathologic conditions. Sometimes it produces progressive systemic disease in debilitated or immunosuppressed patients, particularly if cell-mediated immunity is impaired. Sepsis may occur in patients with compromised cellular immunity, e.g., those undergoing cancer chemotherapy or those with lymphoma, AIDS, or other conditions. *Candida* may produce bloodstream invasion,

thrombophlebitis, endocarditis, or infection of the eyes and virtually any organ or tissue when introduced intravenously, e.g., via tubing, needles, narcotics abuse, etc.

*Candida albicans* has been shown to be diploid with balanced lethals, and therefore probably does not go through a sexual phase or meiotic cycle. This yeast  
5 appears to be able to spontaneously and reversibly switch at high frequency between at least seven general phenotypes. Switching has been shown to occur not only in standard laboratory strains, but also in strains isolated from the mouths of healthy individuals.

Nystatin, ketoconazole, and amphotericin B are drugs which have been used to treat oral and systemic *Candida* infections. However, orally administered nystatin is  
10 limited to treatment within the gut and is not applicable to systemic treatment. Some systemic infections are susceptible to treatment with ketoconazole or amphotericin B, but these drugs may not be effective in such treatment unless combined with additional drugs. Amphotericin B has a relatively narrow therapeutic index and numerous undesirable side effects and toxicities occur even at therapeutic concentrations. While ketoconazole and  
15 other azole antifungals exhibit significantly lower toxicity, their mechanism of action, inactivation of cytochrome P<sub>450</sub> prosthetic group in certain enzymes, some of which are found in humans, precludes use in patients that are simultaneously receiving other drugs that are metabolized by the body's cytochrome P<sub>450</sub> enzymes. In addition, resistance to these compounds is emerging and may pose a serious problem in the future.

20 There is a need in the art for an effective treatment of opportunistic infections caused by *Candida albicans*. Therefore, one object of the invention is to provide screening assays for identifying potential inhibitors of *Candida albicans* growth. Another object of the invention is to provide screening assays and to identify potential inhibitors of *Candida albicans* growth that are based on inhibition of transcription in this  
25 organism.

Synthesis of mRNA in eukaryotes requires RNA polymerase II and accessory transcription factors, some of which are general and act at most, if not all, promoters, and others of which confer specificity and control. Five general factors, a, b, d, e, and g, have been purified to homogeneity from the yeast *S. cerevisiae*, and have  
30 been identified as counterparts of human or rat factors, TFIIE, TFIIH, TFIID, TFIIB and TFIIF, respectively. These factors assemble at a promoter in a complex with RNA polymerase II to initiate transcription. Binding studies have shown that the order of assembly of the initiation complex on promoter DNA begins with factor d (TFIID), is

followed by factor e (TFIIB), and then by polymerase and the remaining factors. Factors b (TFIIH), e (TFIIB) and g (TFIIF), however, bind directly to polymerase II, and as many as four of the five factors may assemble with the polymerase in a holoenzyme before promoter binding. The functional significance of interactions revealed by binding studies is not clear in that only a few percent of initiation complexes may give rise to transcripts.

Many aspects of transcription by RNA polymerase II are conserved between yeast and higher eukaryotes. For example, there is extensive amino acid sequence similarity among the largest subunits of the yeast, *Drosophila* and mammalian polymerases. Other components of the transcription apparatus, such as TATA-binding and enhancer binding factors, are in some instances interchangeable between yeast and mammalian *in vitro* binding or transcription systems. There are, nonetheless, significant differences between the two systems. TATA elements are located from 40 to 120 or more base pairs upstream of the initiation site of an *S. cerevisiae* promoter, and where these elements occur, they are required for gene expression. The fact that *C. albicans* genes function in *S. cerevisiae* suggests that it also uses the 40 to 120 base pair spacing between the TATA element and initiation site. In contrast, mammalian (as well as *S. pombe*) TATA elements and transcription start sites are only 25 to 30 bp apart, and deletion of a TATA element does not always reduce the frequency of transcription initiation, although it may alter the initiation site. There are also varying degrees of homology between transcription factor sequences from yeast and mammalian sources. Some of the multisubunit factors, such as RNA polymerase II, TFIIF, and TFIID, contain different numbers of subunits in humans and yeast. The molecular weights of corresponding polypeptides differ between humans and yeast, with sequences being found in a given yeast factor not being found in its human counterpart and vice versa.

TATA-binding protein (TBP) is the central initiation factor for transcription by all three nuclear RNA polymerases, and is highly conserved throughout the eukaryotic kingdom. The 180 amino acid carboxy-terminal core domain is sufficient for TATA element binding, for all essential functions in *S. cerevisiae*, and is 80% identical between *S. cerevisiae* and humans. *In vitro*, yeast and human TBPs can functionally replace one another in terms of basal RNA polymerase II transcription, and they display nearly identical DNA sequence requirements for TATA elements. However, TBP exhibits species-specific behavior *in vivo*. For example, human and yeast TBP's are not species

interchangeable in supporting cell growth (Gill and Tjian, Cell 65:333-340, (1991); Cormack et al., Cell 65:341-348 (1991)). Human and *S. cerevisiae* TFIIB's have 50-60% amino acid sequence identity, and also are not species interchangeable in supporting cell growth.

5           Operative substitution of the same transcription factor in transcription systems of different yeast species is not predictable. This is true despite a high degree of amino acid sequence identity among some transcription factors from different yeast species. For example, the ability of a given transcription factor to support efficient and accurate transcription in a heterologous yeast species is not predictable. Li et al. (1994, 10 Science 263:805) tested the interchangeability of *S. cerevisiae* and *S. pombe* transcription factors *in vitro*, and report that many *S. cerevisiae* components cannot substitute individually for *S. pombe* RNA transcription factors a, e, or polymerase II, but some combinations of these components were effective. In one instance, active transcription could not be reconstituted when *S. cerevisiae*-derived TFIIB was the sole substitution into 15 a TFIIB-depleted set of factors from *S. pombe*. A TFIIB-RNA polymerase II combination from *S. cerevisiae* was able to substitute, indicating that the functional interaction of these two components is not only important, but also that the activity may be dependent on species-specific determinants that cannot be complemented by either component derived from a different organism. The unpredictability in making substitutions of a given factor 20 among different yeast species is also evident in that such substitutions are not reciprocal; that is, substitutions of *S. pombe* fractions into an *S. cerevisiae* transcription system are less effective than the reverse substitutions (Li et al., *supra*).

The yeast *Candida albicans* differs from most yeast strains in that it does not use the same genetic code that most organisms, whether mammalian or yeast, utilize. 25 Santos et al. (1995, Nucleic Acids Research, 23:1481) report that the codon CUG, which in the universal code is read as a leucine, is decoded as a serine in *Candida*. Therefore, any CUG codon that is decoded in *Candida albicans* as a serine, would be decoded as a leucine in the transformed *S. cerevisiae*. Any gene containing a CUG codon would therefore be translated as different amino acid sequences in *Candida albicans* and *S.* 30- *cerevisiae*. Such mistranslation may produce an inactive protein, since the amino acids serine and leucine have markedly different chemical properties and serine is known to be an essential residue in the active site of some enzymes. Replacement of leucine by serine

at CUG encoded residues is a serious problem in the use of many reporter systems (e.g.  $\beta$ -galactosidase, Chloramphenicol acetyltransferase, Flux) in Candida albicans. Previous experiments have shown that translation by *Candida* of CUG as serine instead of leucine often resulted in the production of inactive reporter proteins.

5 Another object of the invention is to provide an assay for screening for selective inhibition of Candida albicans growth and/or viability.

Yet another object of the invention is to provide a molecular target for inhibition of Candida albicans transcription or transcription initiation.

## 10 SUMMARY OF THE INVENTION

The invention encompasses a recombinant nucleic acid comprising a nucleic acid sequence encoding Candida albicans TBP.

The invention also encompasses a vector comprising a nucleic acid sequence encoding Candida albicans TBP, and a transformed host cell containing a nucleic acid  
15 sequence encoding Candida albicans TBP.

The invention also encompasses a method for producing recombinant Candida albicans TBP, comprising culturing a host cell transformed with a nucleic acid encoding Candida albicans TBP under conditions sufficient to permit expression of the nucleic acid encoding Candida albicans TBP, and isolating Candida albicans TBP.

20 The invention also encompasses a screening method for identifying an inhibitor of Candida albicans growth, comprising detecting inhibition of mRNA transcription in an *in vitro* transcription assay comprising a DNA template, RNA polymerase II, recombinant Candida albicans TBP, and a candidate inhibitor, wherein production of an mRNA transcript complementary to the DNA template occurs in the  
25 absence if the candidate inhibitor.

The invention also encompasses a screening method for identifying an inhibitor of Candida albicans growth, comprising detecting in the presence of a candidate inhibitor inhibition of formation of a complex comprising a DNA template and recombinant Candida albicans TBP, wherein in the absence of the candidate inhibitor,  
30 formation of the complex occurs. The method also may be performed in the presence of additional factors, such as TFIIB, RNA polymerase II and TFIIF.

The invention also encompasses a screening method for identifying an

inhibitor of Candida albicans growth, comprising detecting in the presence of a candidate inhibitor, inhibition of formation of a complex comprising Candida albicans TFIIB and Candida albicans TBP, wherein in the absence of the candidate inhibitor formation of the complex occurs. Preferably, the complex will include a DNA template.

5 The invention also encompasses a screening method for identifying an inhibitor of Candida albicans growth, comprising detecting in the presence of a candidate inhibitor inhibition of formation of a complex comprising RNA polymerase II, Candida albicans TBP, and Candida albicans TFIIB, wherein in the absence of the candidate inhibitor formation of the complex occurs. Preferably, the complex will include a DNA  
10 template and the RNA polymerase II from *C. albicans*.

In the above-described screening methods, detection may be performed in the presence of a plurality of candidate inhibitors. In screening methods of the invention which involve screening of a plurality of candidate inhibitors, the plurality of inhibitors may be screened together in a single assay or individually using multiple simultaneous  
15 individual detecting steps.

The invention also encompasses a method of preventing Candida albicans growth in culture, comprising contacting the culture with an inhibitor that selectively inhibits the biological activity of Candida albicans TBP.

The invention also encompasses a method of preventing Candida albicans  
20 growth in a mammal, comprising administering to a mammal a therapeutically effective amount of an inhibitor that inhibits the biological activity of Candida albicans TBP.

As used herein, "inhibition" refers to a reduction in the parameter being measured, whether it be Candida albicans growth or viability, Candida albicans TBP-mediated transcription, or formation of a Candida albicans TBP transcription complex.  
25 The amount of such reduction is measured relative to a standard (control). Because of the multiple interactions of Candida albicans TBP in transcription initiation, the target product for detection varies with respect to the particular screening assay employed. Three preferred detection products presented in this disclosure are; a) newly transcribed mRNA, b) a DNA-TBP complex, and c) a TBP-TFIIB-RNA polymerase II complex.  
30 "Reduction" is defined herein as a decrease of at least 25% relative to a control, preferably of at least 50%, and most preferably of at least 75%.

As used herein, "growth" refers to the normal growth pattern of Candida

albicans, i.e., to a cell doubling time of 60 - 90 minutes. "Viability" refers to the ability of Candida albicans to survive in culture for 48 hours.

"Biological activity" refers to the ability of TBP to form a transcription complex with a DNA template or other proteins of the transcription complex, or to  
5 interact with other transcription components so as to permit initiation of transcription.

"DNA template" refers to double stranded DNA and, where indicated by the particular binding assay to single stranded DNA, at least 10 nucleotides in length, that may be negatively supercoiled if double-stranded, possesses a promoter region, and contains a yeast TATA consensus region. DNA templates useful herein preferably will  
10 contain a TATA sequence that is located from 40 to 120 or more base pairs upstream of the initiation site (distance measured from the first T of the TATA element to the 5'-most initiation site). An especially efficient DNA template for use in methods of the invention involving transcription is devoid of guanosine residues, and therefore a "G-minus" or "G-less" cassette is preferred.

15 "mRNA transcript" refers to a full-length transcript as well as to truncated transcripts, oligonucleotide transcripts and dinucleotide RNAs.

"Formation of a complex" refers to the binding of TBP to other transcription factors (i.e., protein-protein binding) as well as to binding of TBP to a DNA template; such binding will, of course, be a non-covalent association.

20 Other features and advantages of the invention will be apparent from the description, preferred embodiments thereof, the drawings, and from the claims.

### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 presents the nucleotide and amino acid sequences of the Candida  
25 albicans transcription factor TBP.

Fig. 2 presents nucleotide and amino acid sequence of the Candida albicans transcription factor TFIIB.

### DESCRIPTION

30 The invention is based on the discovery of a novel protein, Candida albicans TBP, and on the isolation of recombinant DNA encoding Candida albicans transcription factor TBP. Because TBP is essential for viability of the cell, a compound that blocks the biological activity of the protein is expected to have fungicidal properties.

Therefore, the invention is also based on the development of assays for screening from inhibitors of TBP.

Isolation and Characterization of the *Candida albicans* TBP Gene

Given the unpredictability with respect to operative substitutions of a given transcription factor among different yeast strains, one cannot assume that strategies for cloning of the gene encoding a given transcription factor which are based on factor function, such as genetic complementation, will work. Other cloning strategies, which do not require functional complementation, such as those based on homology at the nucleic acid level, may be utilized in an attempt to circumvent a requirement for factor function. For example, Southern hybridization of specific sequences to a library carried in *E. coli* and PCR amplification of potentially highly homologous regions of a gene are two strategies that have been successfully used to clone homologous genes from different organisms.

The approach used to clone the *Candida albicans* homolog of TBP involved genetic complementation of mutant *S. cerevisiae* strains. A library of *Candida albicans* genomic sequences was introduced into a strain of *S. cerevisiae* that contained a mutated TBP gene (*spt15*). This mutant strain was capable of growth at 30° C, but was non-viable at 37° C, due to a temperature sensitive mutation in the TBP gene. Following transformation of the library into the strain, the cells were grown at 37° C, and the colonies which grew at this non-permissive temperature were further studied as potentially carrying a *Candida albicans* homolog of the defective gene. This approach will only work if a *Candida albicans* homolog is able to substitute functionally *in vivo* for the defective gene.

After candidate clones were isolated by growth at the nonpermissive temperature, the library plasmid DNA was recovered from the cell and retested to confirm that the *C. albicans* sequences on the plasmid were substituting for the *S. cerevisiae* gene. Subclones of the *C. albicans* sequences were constructed by standard cloning methods, and the minimal *Candida* DNA sequences that substituted were sequenced using standard methods.

The nucleotide sequence encoding *Candida albicans* TBP and the predicted amino acid sequence of the encoded protein are presented in Fig. 1 (SEQ ID NOS: 1 and 2). The nucleotide sequence encoding *Candida albicans* TFIIB and the predicted amino

acid sequence of the encoded protein are presented in Fig. 2 (SEQ ID NOS: 3 and 4).

#### Methods For Screening Potential Inhibitors of *Candida albicans* Growth and/or Viability

Because TBP initiation factor is essential for transcription initiation, the  
5 recombinant *Candida albicans* TBP gene and recombinant protein encoded by this gene  
may be utilized in screening assays for inhibitors of *Candida albicans* growth and  
viability. The screening assays of this invention detect inhibition of the *Candida albicans*  
TBP-mediated component of transcription initiation, either by measuring inhibition of  
transcription, transcription initiation, or initiation complex formation, or by assaying  
10 formation of a protein/DNA or a protein/protein complex.

#### EXAMPLE 1

##### Screening for Inhibitors of Transcription

###### a) Transcription Assay Components.

15 An *in vitro* transcription assay consisting of the minimal components  
necessary to synthesize an mRNA transcript from a DNA template can be used to screen  
for inhibition of mRNA production. The elements of such an assay consist of; a) a DNA  
template, b) RNA polymerase II, c) recombinant *Candida albicans* TBP, and d) a TFIIB  
which is preferably *Candida albicans* TFIIB. In order to increase the efficiency of  
20 transcription, additional components of the transcription complex may be included, as  
desired; e.g., TFIIE, TFIIF, TFIIH, etc.

Parvin and Sharp (*Cell* 73, 533-540, 1993) have reconstituted gene  
transcription *in vitro* with a minimal reaction containing a DNA template, RNA  
polymerase II, TFIIB, and TBP. For efficient transcription under minimal conditions, the  
25 DNA template (a) is supercoiled, and (b) possesses a promoter region containing a TATA  
consensus region. Additionally, Lue et al. (*Science* 246, 661-664, 1989) have determined  
that transcription may be detected most efficiently with a DNA template devoid of  
guanosine residues (a G-minus or G-less cassette). Promoter dependence is demonstrated  
by the loss of signal when a plasmid lacking promoter sequences is utilized as a template.  
30 Correct initiation is demonstrated by the production of a band with a mobility consistent  
with the size of the expected product on denaturing polyacrylamide electrophoresis gels.

As stated above, *Candida albicans* TBP forms a transcription initiation  
complex with RNA polymerase II. Therefore, it is desired that an *in vitro* transcription

assay according to the invention contain RNA polymerase II. Although it is possible to perform an inhibitor screening assay using RNA polymerase II from a yeast strain other than *Candida albicans*, e.g., *S. cerevisiae*, it is most desirable to use a homologous assay in which the transcription complex components are from *Candida albicans*.

5 A method for *S. cerevisiae* RNA polII purification is described in Edwards et al. (*Proc. Natl. Acad. Sci. USA* 87: 2122-2126 (1990)). Alternatively, highly purified RNA polymerase II from *Candida albicans* was provided as follows.

RNA polymerase II activity was measured in reactions containing 50 mM Tris-Cl, pH 7.9 (4° C), 50 mM (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 2.5 mM MnCl<sub>2</sub>, 0.1 mM EDTA, 5 mM  
10 DTT, 100 µg/ml BSA, 0.6 mM ATP, CTP and GTP, 25 µM UTP (2.5 µCi) [ $\alpha$ -<sup>32</sup>P] UTP and 100 µg/ml heat-denatured calf thymus DNA in a final volume of 50 µl. Reactions were incubated for 60 min. at 30° C and terminated by addition of 50 µl 15% (w/v) trichloroacetic acid. Acid-insoluble radioactivity was collected by filtration through glass fiber filters and quantified by liquid scintillation spectrophotometry. One unit of RNA  
15 polymerase activity catalyzes the incorporation of 1 pmol of UTP into acid-insoluble material in 60 min. under the conditions described above.

*Candida albicans* was obtained from the American Type Culture Collection (ATCC 10231) and cultured in YPD medium (Current Protocols in Molecular Biology, Vol. 2, 13, Suppl. 19 (1989)) at 30° C with vigorous agitation and aeration. All  
20 procedures were carried out at 4° C using 18 liter cultures. Cells were harvested by centrifugation (5000 rpm, 10 min., Sorvall H6000 rotor), washed once with ~ 1l ice-cold deionized water and repelleted as above. The cell pellet (200-300 g wet weight) was thoroughly resuspended in a volume of Buffer A (50 mM Tris-HCl, pH 7.9, 4° C, 10% glycerol, 1 mM EDTA, 5 mM MgCl<sub>2</sub>, and protease inhibitor) containing 300 mM (NH<sub>4</sub>)<sub>2</sub>  
25 SO<sub>4</sub> equivalent to the packed volume of cells (determined by weight assuming a density of 1 g/ml cells). Resuspended cells were either processed immediately as described below or frozen by pipetting into liquid N<sub>2</sub> and stored at -80 C. Frozen cells were thawed on ice prior to proceeding. Following the addition of NP-40 to a final concentration of 0.1%, cells were disrupted by grinding with 1 ml acid-washed glass beads/ml cell  
30- suspension (Sigma, 400-625 µM) using 12 bursts of 30 sec. each in a Bead Beater (BioSpec). Glass beads were allowed to settle out and the supernatant was centrifuged at 30,000 x g for 40 min. Solid (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> was slowly added to a final concentration of 0.4 g/ml supernatant and the resulting precipitate was pelleted by centrifugation at 100,000

x g for 30 min. The pellet was resuspended with a volume of Buffer A sufficient to yield a conductivity equivalent to Buffer A containing 75 mM  $(\text{NH}_4)_2 \text{SO}_4$ .

Following centrifugation of the resuspension at 10,000 x g for 10 min, this supernatant (~ 1- 1.5 mg protein/ml) was loaded onto a 300 ml DE-52 DEAE-cellulose column equilibrated with Buffer A containing 75 mM  $(\text{NH}_4)_2 \text{SO}_4$ . After washing with 5 column volumes Buffer A containing 75 mM  $(\text{NH}_4)_2 \text{SO}_4$ , and 5 column volumes Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ , RNA polymerase II was eluted with 5 column volumes Buffer A containing 0.4 M  $(\text{NH}_4)_2 \text{SO}_4$ . Fractions were collected containing the peak of protein, determined by absorbance at 280 nm and pooled. The pool was dialyzed against Buffer A containing 20% glycerol for 3 hr. at 4° C.

The 0.4 M  $(\text{NH}_4)_2 \text{SO}_4$  eluate from DEAE-cellulose (261 mg protein, 290 ml) was diluted with sufficient Buffer A to lower the conductivity to the equivalent of Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ , centrifuged at 10,000 x g for 10 min. and the supernatant was loaded at a flow rate of 30 ml/hr onto a 30 ml DEAE-cellulose column equilibrated with Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ . After washing with 3 column volumes of Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ , the column was developed with a 200 ml linear gradient of 0.15 - 0.4 M  $(\text{NH}_4)_2 \text{SO}_4$  in Buffer A at a flow rate of 45 ml/hr. Fractions from the single peak of amanitin-sensitive RNA polymerase activity, eluting around 0.22 M  $(\text{NH}_4)_2 \text{SO}_4$ , were pooled (21.1 mg protein, 45 ml) and loaded directly onto a 5 ml Heparin agarose column equilibrated with Buffer A containing 0.2 M  $(\text{NH}_4)_2 \text{SO}_4$ . The column was washed with 3 column volumes of Buffer A containing 0.2 M  $(\text{NH}_4)_2 \text{SO}_4$  and developed with an 80 ml linear gradient of 0.2 - 0.6 M  $(\text{NH}_4)_2 \text{SO}_4$  in Buffer A. The active fractions, which eluted at approximately 0.42 M  $(\text{NH}_4)_2 \text{SO}_4$  were pooled (2.0 mg protein, 15 ml), frozen in 300  $\mu\text{l}$  aliquots in liquid  $\text{N}_2$ , and stored at -80° C where activity was stable for at least 6 months.

Purification of protein initiation factors used in the assay is accomplished by standard methods known in the art (e.g., phosphocellulose chromatography followed by gel filtration), as described in (*Nature* 346, 387-390 (1990)).

To screen for *Candida albicans* TBP-mediated transcription inhibition, a transcription assay is reconstituted using recombinant *Candida albicans* TBP. Supercoiled plasmid DNA containing the CYC1 promoter linked to the G-less cassette described by Lue *et al.* (*Science* 246, 661-664 (1989)), is purified by standard methods for purification of supercoiled circular DNA (Current Protocols in Molecular Biology,

Vol. 2, 13, Suppl. 19 (1989)). 10 - 100 ng of *Candida albicans* TFIIB, 10 - 100 ng of *Candida albicans* TBP, 10 - 100 ng *Candida albicans* RNA polymerase II and 1  $\mu$ g plasmid DNA are added to 50  $\mu$ l reaction mixtures containing 50 mM HEPES, pH 7.5, 10% glycerol, 90 mM potassium glutamate, 0.75% polyethylene glycol (molecular weight 3350), 10 mM magnesium acetate, 5 mM EGTA, 5 mM DTT, 0.4 mM ATP, 0.4 mM CTP, 10  $\mu$ M [ $\alpha$ - $^{32}$ P]UTP, 0.2 mM 3'-O-methyl-GTP, and containing or lacking a candidate inhibitor molecule. Reactions are incubated at 30 $^{\circ}$  C for 30 - 60 min. and RNA synthesis is detected as described below.

b) Detection of Transcribed RNA.

The detection of newly transcribed RNA is achieved by standard methods (Current Protocols in Molecular Biology, Vol. 1, 4.10, Suppl 24 (1989)). As one example, RNA synthesis can be detected as incorporation of a radioactively or fluorescently labeled nucleotide into higher molecular weight RNA products, determined by one of the following methods: 1) acid-insoluble labeled material quantitated by the appropriate method (e.g. scintillation counting for radioactive precursors, fluorometry for fluorescent precursors); 2) labeled reaction product that hybridizes to oligonucleotides complementary to the correctly initiated transcript (i.e., northern blot analysis); 3) the presence of a labeled band with the appropriate mobility detected by autoradiography, on denaturing polyacrylamide electrophoresis gels; 4) any other method that discriminates mononucleotides from polynucleotides, where polynucleotides are the desired RNA product. Such methods may utilize one or more well known techniques of molecular biology (Current Protocols in Molecular Biology, Vol. 2, 13, Suppl. 19 (1989)), for example; UV analysis; affinity systems (e.g., affinity chromatography, nitrocellulose filtration, biotin/streptavidin systems, immunoaffinity,) (Current Protocols in Molecular Biology, Vol. 2, 13, Suppl. 19 (1989)); and high performance liquid chromatography.

The inclusion of an inhibitor molecule that interferes with *Candida albicans* TBP biological activity inhibits transcription. In this assay inhibition is measured as a reduction in the amount of mRNA transcript produced relative to the amount of mRNA transcript produced in the absence of the inhibitor (the positive control). A decrease in amount of mRNA transcript is indicative of an inhibitor. The determination of effective levels of mRNA transcript inhibition is described below.

## EXAMPLE 2

### Screening for Inhibition of DNA-Protein Complex Formation

A DNA-protein binding assay consisting of the minimal components necessary to permit DNA-Candida albicans TBP binding to occur can be used to screen  
5 for inhibition of the formation of the DNA-Candida albicans TBP complex during transcription initiation. The essential elements of such an assay consist of; a) a DNA template, b) recombinant Candida albicans TBP, and optionally c) a candidate Candida albicans TBP inhibitor.

The inclusion of an inhibitor molecule that interferes with the interaction  
10 between the Candida albicans TBP and the DNA template inhibits transcription initiation. The inhibitor may interact directly with the Candida albicans TBP protein, and/or it may interact with the DNA template at the DNA site of Candida albicans TBP binding. In this assay inhibition is measured as a reduction in the amount of DNA- Candida albicans TBP complex produced relative to the amount of DNA- Candida albicans TBP complex  
15 produced in the absence of the inhibitor (the positive control). A decrease in the amount of DNA- Candida albicans TBP complex is indicative of an inhibitor. Determination of effective levels of DNA- Candida albicans TBP inhibition is described below.

One DNA binding assay is constructed as follows. 10 - 100 ng Candida albicans TBP, expressed in and purified from *E. Coli* as described above, is incubated  
20 with 0.5 ng labeled (e.g. radioactively or fluorescently labeled) oligonucleotide containing a TATA element such as the one described by Buratowski *et al.* (*Cell* 56, 549-561 (1989)) in reactions containing 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT), 100 µg/ml BSA, 5 - 20 µg/ml poly (dG-dC):(dG-dC) and a candidate inhibitor of complex formation. Reactions are incubated at  
25 30° C for 30-60 min.

Formation of a DNA-TBP complex may be detected as retention of labeled DNA (the label being detected by an appropriate methodology such as scintillation counting for radiolabeled DNA or fluorometry for fluorescently labeled DNA) utilizing known affinity methods for protein immobilization (e.g., biotin/streptavidin, nitrocellulose  
30 filtration, affinity chromatography, immunoaffinity). Nonretention of labeled DNA due to the failure of Candida albicans TBP-DNA complex formation is indicative of an effective inhibitor.

Complex formation may also be detected as retention of labeled *Candida albicans* TBP (e.g. radioactively, fluorescently) utilizing known methods for immobilizing DNA. Nonretention of labeled *Candida albicans* TBP due to the failure of *Candida albicans* TBP-DNA complex formation is indicative of an effective inhibitor. These methods are suitable for high-throughput chemical compound library screening applications such as those commonly used in drug discovery.

A third example of detecting DNA/protein complex formation involves detection of an electrophoretic mobility shift of labeled DNA on 4% polyacrylamide gels containing 5% (v/v) glycerol, 25 mM Tris, 100 mM glycine, 1mM EDTA, 5 mM MgCl<sub>2</sub>, pH 8.3 in the presence of *Candida albicans* TBP. The position of the labeled oligonucleotide is detected by appropriate methods (e.g., autoradiography for radioactive oligonucleotide). The absence or deviation of the expected mobility shift due to DNA-*Candida albicans* TBP complex formation is indicative of an effective inhibitor.

Finally, other methods for detecting or separating DNA-protein complexes may be used, including UV crosslinking analysis, high performance liquid chromatography, phage display technology (U.S. Patent No. 5,403,484. Viruses Expressing Chimeric Binding Proteins), fluorescence polarization, and surface plasmon resonance (Biacore, Pharmacia Biosensor, North America) as described below.

### 20 EXAMPLE 3

#### Screening for Inhibition of DNA-Protein Complex Formation

A DNA-protein binding assay consisting of the minimal components necessary to permit DNA-*Candida albicans* TBP association to occur can be used to screen for inhibition of the formation of the DNA-TBP-*Candida albicans* TFIIB complex during transcription<sup>4</sup> initiation. The components of such an assay include: a) a DNA template, b) recombinant *Candida albicans* TBP, c) TFIIB, preferably from *C. albicans*, and optionally d) a candidate *Candida albicans* TFI inhibitor.

The inclusion of an inhibitor molecule that interferes with the interaction between the *Candida albicans* TBP and the DNA template inhibits transcription initiation. The inhibitor may interact directly with the *Candida albicans* TBP protein, and/or it may interact with TFIIB and/or with the DNA template at the site of TFIIB/TBP binding. In this assay inhibition is measured as a reduction in the amount of DNA-TBP-TFIIB complex produced relative to the amount of DNA-TBP-TFIIB complex produced in the

absence of the inhibitor (the positive control). A decrease in the amount of DNA-TBP-TFIIB complex is indicative of an inhibitor. Determination of effective levels of DNA-TBP-TFIIB inhibition is described below.

One DNA binding assay is constructed as follows. 10 - 100 ng *Candida albicans* TBP, expressed in and purified from *E. Coli* as described above, is incubated with 0.5 ng labeled (e.g. radioactively or fluorescently labeled) oligonucleotide containing a TATA element such as the one described by Buratowski *et al.* (*Cell* 56, 549-561 (1989) and 10 - 100 ng *Candida albicans* TFIIB in reactions containing 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT), 100 µg/ml BSA, 5 - 20 µg/ml poly (dG-dC):(dG-dC) and a candidate inhibitor of complex formation. Reactions are incubated at 30° C for 30-60 min.

Formation of a DNA-TBP-TFIIB complex may be detected as retention of labeled DNA (the label being detected by an appropriate methodology such as scintillation counting for radiolabeled DNA or fluorometry for fluorescently labeled DNA) utilizing known affinity methods for protein immobilization (e.g., biotin/streptavidin, nitrocellulose filtration, affinity chromatography, immunoaffinity). Nonretention of labeled DNA due to the failure of *Candida albicans* TFIIB-TBP-DNA complex formation is indicative of an effective inhibitor.

Complex formation may also be detected as retention of labeled *Candida albicans* TBP (e.g. radioactively, fluorescently) utilizing known methods for immobilizing DNA. Nonretention of labeled *Candida albicans* TBP due to the failure of *Candida albicans* TFIIB-TBP-DNA complex formation is indicative of an effective inhibitor. The preceding two methods are suitable for high-throughput chemical compound library screening applications such as those commonly used in drug discovery.

A third example of detecting DNA/protein complex formation involves detection of an electrophoretic mobility shift of labeled DNA on 4% polyacrylamide gels containing 5% (v/v) glycerol, 25 mM Tris, 100 mM glycine, 1mM EDTA, 5 mM MgCl<sub>2</sub>, pH 8.3 in the presence of *Candida albicans* TFIIB and TBP. The position of the labeled oligonucleotide is detected by appropriate methods (e.g., autoradiography for radioactive oligonucleotide). The absence or deviation of the expected mobility shift due to DNA-*Candida albicans* TBP complex formation is indicative of an effective inhibitor.

Finally, other methods for detecting or separating DNA-protein complexes may be used, including UV crosslinking analysis, high performance liquid

chromatography, phage display technology (U.S. Patent No. 5,403,484. Viruses Expressing Chimeric Binding Proteins), and surface plasmon resonance (Biacore, Pharmacia Biosensor, North America) as described below.

#### 5 EXAMPLE 4

##### Screening for Inhibition of Protein-Protein Complex Formation

A protein-protein binding assay consisting of the minimal components necessary to permit Candida albicans TBP-Candida albicans TFIIB binding to occur can be used to screen for inhibition of the formation of the Candida albicans TBP-Candida  
10 albicans TFIIB complex during transcription initiation. The elements of such an assay consist of; a) recombinant Candida albicans TBP, b) TFIIB, preferably a recombinant Candida albicans TFIIB, and optionally c) a candidate inhibitor of binding.

The inclusion of an inhibitor molecule that interferes with the interaction between the Candida albicans TBP and Candida albicans TFIIB inhibits transcription  
15 initiation. The inhibitor may interact with the Candida albicans TBP or TFIIB protein and thus induce a conformational change which prevents binding, or it may directly inhibit the interaction of Candida albicans TFIIB and TBP proteins. In this assay, inhibition is measured as a reduction in the amount of Candida albicans TBP-TFIIB complex produced relative to the amount of Candida albicans TBP-TFIIB complex produced in the absence  
20 of the inhibitor (the positive control). A decrease in the amount of TFIIB-TBP complex is indicative of an inhibitor. Determination of effective levels of inhibition of Candida albicans TBP-TFIIB binding is described below.

One assay for formation of Candida albicans TBP-TFIIB complex is provided as follows. 10 - 100 ng Candida albicans TFIIB and 10 - 100 ng Candida  
25 albicans TBP are expressed in and purified from *E. coli* as described above, and are added to reactions containing 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT) 100 µg/ml BSA, and a candidate inhibitor. The mixture is then incubated at 30° C for 30 - 60 min.

Formation of a complex comprising Candida albicans TBP and Candida  
30 albicans TFIIB may be detected by an electrophoretic mobility shift of labeled (e.g. radioactive or fluorescent) TBP or TFIIB on 4% polyacrylamide gels containing 5% (v/v) glycerol, 25 mM Tris, 100 mM glycine, 1mM EDTA, 5 mM MgCl<sub>2</sub>, pH 8.3 in the

presence of the unlabeled partner. The position of the labeled partner is detected by appropriate methods (e.g., autoradiography for radioactive oligonucleotide). The absence or deviation of the expected mobility shift due to Candida albicans TFIIB-TBP complex formation is indicative of an effective inhibitor.

5                    Formation of a complex comprising Candida albicans TBP and Candida albicans TFIIB may be detected as retention of labeled TBP utilizing known affinity methods for immobilizing the Candida albicans TFIIB protein (e.g., biotin/streptavidin, nitrocellulose filtration, affinity chromatography, immunoaffinity). The failure of formation of the Candida albicans TFIIB-TBP complex is indicative of inhibition, and is  
10 indicated by nonretention of labeled TBP. Alternatively, the immobilized element may be Candida albicans TBP and the labeled partner Candida albicans TFIIB.

                  In the above example, a stronger signal may be conferred in the presence of both TBP and TFIIB and, in addition, a DNA template containing a TATA element. The complex is then quantitated by autoradiography, Phosphorimager technology, or  
15 scintillation counting for radioactively labeled factors, fluorometry for fluorescently labeled factors, luminometry for factors labeled with ligands that are detected using chemiluminescent or phosphorescent probing methodologies, or other similar detection methods or materials labeled as described above that are standard in the art.

                  Other methods for detecting or separating protein-protein complexes may  
20 be used, including UV crosslinking analysis, high performance liquid chromatography, phage display technology, and surface plasmon resonance as described herein.

#### EXAMPLE 5

##### Assay for Formation of TBP-TFIIB-RNA Polymerase II-DNA Complex

25                    Formation of a TBP, TFIIB, RNA polymerase II, DNA complex is known to be markedly stimulated by the addition of another factor, TFIIF. Previous data indicates that TFIIF from *S. cerevisiae* can function in species as distantly related as *Schizosaccharomyces pombe* and humans, strongly suggesting that this factor can functionally replace its *C. albicans* homolog. Accordingly, this factor is purified from  
30 *S. cerevisiae* by published methods (Sayre, 1992, J. Biol. Chem. 267:23383) and used to reconstitute formation of a complex containing *C. albicans* TBP, TFIIB, RNA polymerase II and promoter containing DNA such as that described for reconstitution of the TFIIB-TBP-DNA complex.

Complex formation is carried out in reactions containing, for example, 10 - 100 ng *Candida albicans* TBP, 10 - 100 ng *Candida albicans* TFIIB, 10 - 100 ng *Candida albicans* RNA polymerase II, 10 - 100 ng *S. cerevisiae* TFIIF, 0.5 ng double-stranded TATA element containing-oligonucleotide (same as that used for TFIIB-TBP-DNA complex assay), 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT), 100 µg/ml BSA, 5 - 20 µg/ml poly (dG-dC); (dG-dC) and compound(s) to be tested for inhibitory activity. Following incubation at 30° C for 30 - 60 min, complexes are detected by one of the methods described above for the TBP-TFIIB-DNA complex. The TBP-TFIIB-RNA polymerase II-DNA complex has a slower electrophoretic mobility than the TBP-TFIIB-DNA complex identified by using the electrophoretic method. In addition, complex formation can be detected as TBP, TFIIB-dependent retention of RNA polymerase II activity (measured by incorporation of labeled nucleotide precursors into acid-insoluble product using the assay for RNA polymerase activity described in the RNA polymerase II purification protocol above) on a matrix with bound TATA-element containing DNA. The IC<sub>50</sub> of inhibitory compounds will be determined by titration into reactions reconstituted as described above. The IC<sub>50</sub> of these compounds against reactions reconstituted with human TBP, TFIIB and RNA polymerase II will also be determined by the same method. Human RNA polymerase II and TFIIF are purified as described previously (Flores et al., 1990, J. Biol. Chem. 265:5629-5634; Reinberg et al., J. Biol. Chem 262:3310-3321). Those compounds whose IC<sub>50</sub> against reactions containing *C. albicans* factors is ≤ 1/5 of their IC<sub>50</sub> against reactions reconstituted with human factors will be tested for their ability to inhibit *C. albicans* growth as described below.

## 25 EXAMPLE 6

### Phage Display Inhibitor Screening

In addition to the above mentioned standard techniques of the art, other technologies for molecular identification can be employed in the identification of inhibitor molecules. One of these technologies is phage display technology (U.S. Patent No. 5,403,484. Viruses Expressing Chimeric Binding Proteins). Phage display permits identification of a binding protein against a chosen target. Phage display is a protocol of molecular screening which utilizes recombinant bacteriophage. The technology involves transforming bacteriophage with a gene that encodes an appropriate ligand (in this case,

a candidate inhibitor) capable of binding to the target molecule of interest. For the purposes of this disclosure, the target molecule may be *Candida albicans* TBP, or a DNA-protein or protein-protein complex formed using TBP and/or TFIIB, as described herein. The transformed bacteriophage (which preferably is tethered to a solid support) express  
5 the candidate inhibitor and display it on their phage coat. The cells or viruses bearing the candidate inhibitor which recognize the target molecule are isolated and amplified. The successful inhibitors are then characterized.

Phage display technology has advantages over standard affinity ligand screening technologies. The phage surface displays the microprotein ligand in a three  
10 dimensional conformation, more closely resembling its naturally occurring conformation. This allows for more specific and higher affinity binding for screening purposes.

### EXAMPLE 7

#### Biospecific Interaction Analysis

15 A second relatively new screening technology which may be applied to the inhibitor screening assays of this invention is biospecific interaction analysis (BIAcore, Pharmacia Biosensor AB, Uppsala, Sweden). This technology is described in detail by Jonsson *et al.* (Biotechniques 11:5, 620-627 (1991)). Biospecific interaction analysis utilizes surface plasmon resonance (SPR) to monitor the adsorption of biomolecular  
20 complexes on a sensor chip. SPR measures the changes in refractive index of a polarized light directed at the surface of the sensor chip.

Specific ligands (i.e., candidate inhibitors) capable of binding to the target molecule of interest (i.e., *Candida albicans* TBP or a protein-protein or protein-DNA complex containing TBP) are immobilized to the sensor chip. In the presence of the  
25 target molecule, specific binding to the immobilized ligand occurs. The nascent immobilized ligand-target molecule complex causes a change in the refractive index of the polarized light and is detected on a diode array. Biospecific interaction analysis provides the advantages of; 1) allowing for label-free studies of molecular complex formation; 2) studying molecular interactions in real time as the assay is passed over the sensor chip;  
30 3) detecting surface concentrations down to 10 pg/mm<sup>2</sup>; detecting interactions between two or more molecules; and 4) being fully automated (Biotechniques 11:5, 620-627 (1991)).

## EXAMPLE 8

### High Throughput Screening of Potential Inhibitors

It is contemplated according to the invention that the screening methods disclosed herein encompass screening of multiple samples simultaneously, also referred to herein as 'high throughput' screening. For example, in high throughput screening, from several hundred to several thousand candidate inhibitors may be screened in a single assay. Several examples of high throughput screening assays useful according to the invention are as follows.

A protein A (pA)-*C. albicans* TBP fusion protein is generated by inserting the coding sequence of TBP in frame downstream of the pA coding sequence of the plasmid pRIT2T (Pharmacia Biotech). The fusion construct is induced, and the resultant recombinant protein is extracted and purified according to the manufacturer's recommended conditions. This procedure can also be carried out for the preparation of a pA-*Candida albicans* TFIIB fusion protein except that the downstream coding sequence is that of TFIIB protein; all other steps would remain the same.

A Dynatech Microlite 2 microtiter plate or equivalent high protein-binding capacity plate is coated with 1  $\mu\text{g}/\text{well}$  human IgG by incubating 300  $\mu\text{l}$  3.33  $\mu\text{g}/\text{ml}$  human IgG (Sigma) in coating buffer (0.2 M sodium carbonate, pH 9.4) in the well for 4-12 hr at 4°C. The coating buffer is then decanted and the wells are washed five times with 300  $\mu\text{l}$  PBS. 300  $\mu\text{l}$  blocking buffer (SuperBlock™ blocking buffer; Pierce) containing 3.33  $\mu\text{g}/\text{ml}$  pA-TBP or pA-TFIIB are added and the plate is incubated for 4 or more hours at 4°C. The plates may be stored in this form at 4°C until ready for use. When ready for use the plates are washed five times with 300  $\mu\text{l}$  PBS. Test compound at a final concentration of 20-200  $\mu\text{M}$ , labeled TBP or TFIIB (i.e., nonfusion protein), whichever is not added during the coating step, and 10 - 1000 fmol promoter-containing oligonucleotides are suspended in HEG buffer containing 200  $\mu\text{g}/\text{ml}$  BSA in a total volume of 150  $\mu\text{l}$  and are added and the reaction is incubated at room temperature with gentle agitation for 60 min. The plate is then washed five times with PBS using a Dynatech plate washer or equivalent. Bound labeled protein is quantitated by adding 250  $\mu\text{l}$  Microscint (Packard) per well and is counted in a microtiter plate-compatible scintillation spectrophotometer.

As an alternative, the protein A fusion and the second, non-fusion protein can be incubated in the presence of test compound in polypropylene microtiter plates

under the same buffer and incubation conditions described above. The reaction mix is then transferred to the wells of a microtiter plate coated with human IgG (which is prepared as described above, and is stored in blocking buffer and is washed five times with 300  $\mu$ l PBS immediately before use) and is incubated for 60 min at room temperature with gentle agitation. Retention of radioactive protein is quantified as above.

Interaction of TBP and TFIIB, which is measured as retention of radioactivity on the plate, is dependent on human IgG coating the plate and wild-type Candida albicans TBP or TFIIB, one of which must be fused to pA. Candidate inhibitors or extracts that inhibit retention of radioactivity by more than 30% are identified and the inhibitory activity is further purified if necessary.

Inhibitors identified as described above are then tested for their ability to inhibit Candida albicans TBP-dependent transcription in an *in vitro* transcription system as described herein, and also may be tested for their ability to inhibit Candida albicans growth.

Other fusion or modified protein systems that are contemplated include, but are not limited to, glutathione-S-transferase, maltose binding protein, influenza virus hemagglutinin, FLAG™ and hexahistidine fusions to Candida albicans TBP or Candida albicans TFIIB which are prepared, expressed, and purified by published methods or biotinylated Candida albicans TBP or TFIIB which are prepared using reactive biotin precursors available commercially. The purified fusion or modified protein is immobilized on a microtiter plate containing the appropriate ligand for each fusion protein (e.g. glutathione, amylose, CA157 antibody, etc., respectively) and the assay is carried out and the results evaluated in essentially the same manner as described above.

## 25 EXAMPLE 9

### Candidate Inhibitors

A "candidate inhibitor," as used herein, is any compound with a potential to inhibit Candida albicans TBP-mediated transcription initiation or complex formation.

A candidate inhibitor is tested in a concentration range that depends upon the molecular weight of the molecule and the type of assay. For example, for inhibition of protein/protein or protein/DNA complex formation or transcription initiation, small molecules (as defined below) may be tested in a concentration range of 1pg - 100 ug/ml, preferably at about 100 pg - 10 ng/ml; large molecules, e.g., peptides, may be tested in

the range of 10 ng - 100 ug/ml, preferably 100 ng - 10 ug/ml.

Inhibitors of *Candida albicans* growth or viability may target the novel transcription factor described herein, TBP, or it may target a protein or nucleic acid that interacts with the novel transcription factor so as to prevent the natural biological interaction that occurs *in vivo* and leads to transcription initiation in *Candida*. Thus, an inhibitor identified as described herein will possess two properties: 1) at some concentration it will inhibit *Candida albicans* growth or viability; and 2) at the same concentration, it will not significantly affect the growth of mammalian, particularly human, cells.

Candidate inhibitors will include peptide and polypeptide inhibitors having an amino acid sequence based upon the novel TBP sequences described herein. For example, a fragment of TBP may act as a competitive inhibitor with respect to TBP binding to other proteins involved in *Candida* transcription, e.g., RNA polymerase II, TFIIB, or with respect to binding of the transcription complex to the DNA template.

Candidate inhibitor compounds from large libraries of synthetic or natural compounds can be screened. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, WI). Combinatorial libraries are available and can be prepared. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g., Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily produceable. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means.

Useful compounds may be found within numerous chemical classes, though typically they are organic compounds, and preferably small organic compounds. Small organic compounds have a molecular weight of more than 50 yet less than about 2,500 daltons, preferably less than about 750, more preferably less than about 350 daltons. Exemplary classes include heterocycles, peptides, saccharides, steroids, and the like. The compounds may be modified to enhance efficacy, stability, pharmaceutical compatibility, and the like. Structural identification of an agent may be used to identify, generate, or

screen additional agents. For example, where peptide agents are identified, they may be modified in a variety of ways to enhance their stability, such as using an unnatural amino acid, such as a D-amino acid, particularly D-alanine, by functionalizing the amino or carboxylic terminus, e.g. for the amino group, acylation or alkylation, and for the carboxyl group, esterification or amidification, or the like. Other methods of stabilization may include encapsulation, for example, in liposomes, etc.

### EXAMPLE 10

#### Measurement for effective inhibition

The amount of inhibition by a candidate inhibitor is quantified using the following formula, which describes reactions reconstituted with a radioactively labeled moiety.

$$\text{Percent Inhibition} = \frac{(\text{CPM}_{\text{Positive Control}} - \text{CPM}_{\text{Sample}})}{(\text{CPM}_{\text{Positive Control}})} \times 100$$

where  $\text{CPM}_{\text{Positive Control}}$  is the average of the cpm in complexes or RNA molecules formed in reactions that lack the candidate inhibitor, and  $\text{CPM}_{\text{Sample}}$  is the cpm in complexes formed in reactions containing the candidate inhibitor. Candidate inhibitors for which the percent inhibition is 50% are titrated into reactions containing either *Candida albicans* TBP or human TBP (expressed in and purified from *E. coli* using existing recombinant clones (Peterson et al., *Science* 248, 1625-1630, 1990; Kao et al., *Science* 248, 1646-1650, 1990; Hoffman, et al., *Nature* 346, 387-390, 1990, and assayed as described above) and their  $\text{IC}_{50}$  with respect to human and *Candida albicans* TBP determined from graphs of compound concentration vs. % inhibition. The  $\text{IC}_{50}$  is defined as the concentration that results in 50% inhibition. Candidate inhibitors for which the  $\text{IC}_{50}$  against *Candida albicans* TBP-containing reactions is less than or equal to 1/5 the  $\text{IC}_{50}$  against human TBP-containing reactions are further tested for their ability to inhibit the growth of *Candida albicans* in culture as described below.

### EXAMPLE 11

#### Measurement for inhibition of *Candida albicans* growth in culture

Once an inhibitor is identified in one or more of the binding or transcription assays described herein, it may be desirable to determine the effect of the inhibitor on the growth and/or viability of *Candida albicans* in culture. A candidate inhibitor is tested for its ability to inhibit growth of *Candida albicans* cells in culture as follows. Methods for performing tests on growth inhibition in culture are well-known in the art. Once such procedure is based on the NCCLS M27P method (The National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; proposed standard, 1992), as follows. Serial dilutions (two- or three-fold steps starting from a maximum concentration of 100 - 200  $\mu\text{g/ml}$ ) of candidate inhibitor are prepared using RPMI-1640 medium as diluent and an aliquot of 100  $\mu\text{l}$  of each dilution is added to the wells of a 96-well polystyrene microtiter plate. Five *Candida albicans* colonies, picked from a Sabouraud Dextrose Agar plate inoculated 14-20 hr previously with the test *Candida albicans* strain (Catalog number 10231 from the American Type Culture Collection Yeast Catalog), are resuspended in RPMI-1640 medium such that the density of cells is 10,000 - 30,000 cells/ml. 100  $\mu\text{l}$  of the cell suspension is added to each of the wells of the 96-well microtiter plate containing diluted candidate inhibitor and medium control. Cultures are mixed by agitation and incubated at 35° C for 48 hr. without agitation, after which cell growth is monitored by visual inspection for the formation of turbidity and/or mycelial colonies. The minimum concentration of candidate inhibitor at which no cell growth is detected by this method is defined as the minimum inhibitory concentration (MIC) for that compound. Examples of MICs for known antifungal compounds obtained using this technique are 0.125 - 0.5  $\mu\text{g/ml}$  for fluconazole and 0.25 - 1.0  $\mu\text{g/ml}$  for amphotericin B (The National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; proposed standard, 1992). An inhibitor identified by the methods described herein, will have MIC which is equivalent to or less than the MICs for fluconazole or amphotericin B.

## EXAMPLE 12

### Transcription Inhibition Counterscreen Using Human TBP

A compound identified as an inhibitor of *Candida albicans* according to one or more of the assays described herein may be tested further in order to determine its effect on the host organism. In the development of useful antifungal compounds for human therapeutics, it is desirable that such compounds act as effective agents in inhibiting the viability of the fungal pathogen while not significantly inhibiting human cell systems. Specifically, inhibitors of *Candida albicans* identified in any one of the above described assays may be counterscreened for inhibition of human TBP.

Recombinant human TBP can be obtained from existing sources and purified by published methods (for example, see Peterson et al., Kao et al., and Hoffman et al., supra) and contacted with the candidate inhibitor in assays such as those described above but using a human system. The effectiveness of a *Candida albicans* TBP inhibitor as a human therapeutic is determined as one which exhibits a low level of inhibition against human TBP relative to the level of inhibition with respect to *Candida albicans* TBP. For example, it is preferred that the amount of inhibition by a given inhibitor of human TBP in a human system be no more than 20% with respect to the amount of inhibition of *Candida albicans* TBP/TFIB in a *Candida* system when tested in any of the assays described above.

### Dosage and Pharmaceutical Formulations

For therapeutic uses, inhibitors identified as described herein may be administered in a pharmaceutically acceptable/biologically compatible formulation, for example, in the form of a cream, ointment, lotion or spray for topical use, or in a physiological solution, such as a salt solution, for internal administration. The amount of inhibitor administered will be determined according to the degree of pathogenic infection and whether the infection is systemic or localized, and will typically be in the range of about 1  $\mu$ g - 100 mg/kg body weight. Where the inhibitor is a peptide or polypeptide, it will be administered in the range of about 100 - 500  $\mu$ g/ml per dose. A single dose of inhibitor or multiple doses, daily, weekly, or intermittently, is contemplated according to the invention.

The route of administration will be chosen by the physician, and may be topical, oral, transdermal, nasal, rectal, intravenous, intramuscular, or subcutaneous.

Budapest Treaty Deposit

*E. coli* transformed with a plasmid containing the gene encoding *Candida albicans* TBP has been deposited in an international depository, the A.T.C.C., Rockville, MD, under the accession number 69900, on September 15, 1995. *E. coli* transformed  
5 with a plasmid containing the gene encoding *Candida albicans* TFIIB has been deposited in an international depository, the A.T.C.C., Rockville, MD, under the accession number 69899, on September 15, 1995. A.T.C.C. Nos. 69900 and 69899 will be available to the public upon the grant of a patent which discloses the accession numbers in conjunction with the invention described herein. The deposits were made under the Budapest Treaty,  
10 will be available beyond the enforceable life of the patent for which the deposit is made, and will be maintained for a period of at least 30 years from the time of deposit and at least 5 years after the most recent request for the furnishing of a sample of the deposit is received by the A.T.C.C. It is to be understood that the availability of these deposits does not constitute a license to practice the subject invention in derogation of patent rights  
15 granted for the subject invention by governmental action.

OTHER EMBODIMENTS

The foregoing examples demonstrate experiments performed and contemplated by the present inventors in making and carrying out the invention. It is believed that these examples include a disclosure of techniques which serve to both apprise  
20 the art of the practice of the invention and to demonstrate its usefulness. It will be appreciated by those of skill in the art that the techniques and embodiments disclosed herein are preferred embodiments only that in general numerous equivalent methods and techniques may be employed to achieve the same result.

All of the references identified hereinabove, are hereby expressly  
25 incorporated herein by reference to the extent that they describe, set forth, provide a basis for or enable compositions and/or methods which may be important to the practice of one or more embodiments of the present inventions.

5

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

10

(i) APPLICANT: SCRIPTGEN PHARMACEUTICALS, INC.

15

(ii) TITLE OF THE INVENTION: NOVEL TATA-BINDING PROTEIN FROM CANDIDA ALBICANS, NUCLEIC ACID SEQUENCE CODING THEREFORE, AND METHODS OF SCREENING FOR INHIBITORS OF CANDIDA ALBICANS GROWTH

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30

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: FastSEQ Version 1.5

35

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

40

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/626,309

(B) FILING DATE: 01-APR-1996

45

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(B) TELEFAX: (212) 753-6237

(C) TELEX:

55

(2) INFORMATION FOR SEQ ID NO:1:

60

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 219 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

65

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 Met Lys Ser Ile Glu Glu Asp Glu Lys Asn Lys Ala Glu Asp Leu Asp  
 1 5 10 15  
 Ile Ile Lys Lys Glu Asp Ile Asp Glu Pro Lys Gln Glu Asp Thr Thr  
 20 25 30  
 Asp Ser Asn Gly Gly Gly Gly Ile Val Pro Thr Leu Gln Asn  
 35 40 45  
 Ile Val Ala Thr Val Asn Leu Asp Cys Arg Leu Asp Leu Lys Thr Ile  
 50 55 60  
 10 Ala Leu His Ala Arg Asn Ala Glu Tyr Asn Pro Lys Arg Phe Ala Ala  
 65 70 75 80  
 Val Ile Met Arg Ile Arg Asp Pro Lys Thr Thr Ala Leu Ile Phe Ala  
 85 90 95  
 15 Ser Gly Lys Met Val Val Thr Gly Ala Lys Ser Glu Asp Asp Ser Lys  
 100 105 110  
 Leu Ala Ser Arg Lys Tyr Ala Arg Ile Ile Gln Lys Leu Gly Phe Asn  
 115 120 125  
 Ala Lys Phe Cys Asp Phe Lys Ile Gln Asn Ile Val Gly Ser Thr Asp  
 130 135 140  
 20 Val Lys Phe Ala Ile Arg Leu Glu Gly Leu Ala Phe Ala His Gly Thr  
 145 150 155 160  
 Phe Ser Ser Tyr Glu Pro Glu Leu Pro Pro Gly Leu Ile Tyr Arg Met  
 165 170 175  
 25 Val Lys Pro Lys Ile Val Leu Leu Ile Phe Val Ser Gly Lys Ile Val  
 180 185 190  
 Leu Thr Gly Ala Lys Lys Arg Glu Glu Ile Tyr Asp Ala Phe Glu Ser  
 195 200 205  
 Ile Tyr Pro Val Leu Asn Glu Phe Arg Lys Asn  
 210 215

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 344 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

50 Met Ser Pro Ser Thr Ser Thr Ala Val Gln Glu Tyr Ile Gly Pro Asn  
 1 5 10 15  
 Leu Asn Val Thr Leu Thr Cys Pro Glu Cys Lys Ile Phe Pro Pro Asp  
 20 25 30  
 Leu Val Glu Arg Phe Ser Glu Gly Asp Ile Val Cys Gly Ser Cys Gly  
 35 40 45  
 55 Leu Val Leu Ser Asp Arg Val Val Asp Thr Arg Ser Glu Trp Arg Thr  
 50 55 60  
 Phe Ser Asn Asp Asp Gln Asn Gly Asp Asp Pro Ser Arg Val Gly Asp  
 65 70 75 80  
 Ala Gly Asn Pro Leu Leu Asp Thr Glu Asp Leu Ser Thr Met Ile Ser  
 85 90 95  
 60 Tyr Ala Pro Asp Ser Thr Lys Ala Gly Arg Glu Leu Ser Arg Ala Gln  
 100 105 110  
 Ser Lys Ser Leu Val Asp Lys Lys Asp Asn Ala Leu Ala Ala Ala Tyr  
 115 120 125  
 65 Ile Lys Ile Ser Gln Met Cys Asp Gly Tyr Gln Leu Pro Lys Ile Val  
 130 135 140  
 Ser Asp Gly Ala Lys Glu Val Tyr Lys Met Val Tyr Asp Glu Lys Pro  
 145 150 155 160

Leu Arg Gly Lys Ser Gln Glu Ser Ile Met Ala Ala Ser Ile Phe Ile  
 165 170 175  
 Gly Cys Arg Lys Ala Asn Val Ala Arg Ser Phe Lys Glu Ile Trp Ala  
 180 185 190  
 5 Lys Thr Asn Val Pro Arg Lys Glu Ile Gly Lys Val Phe Lys Ile Met  
 195 200 205  
 Asp Lys Ile Ile Arg Glu Lys Asn Ala Ala Asn Pro Asn Ala Ala Tyr  
 210 215 220  
 10 Tyr Gly Gln Asp Ser Ile Gln Thr Thr Gln Thr Ser Ala Glu Asp Leu  
 225 230 235 240  
 Ile Arg Arg Phe Cys Ser His Leu Gly Val Asn Thr Gln Val Thr Asn  
 245 250 255  
 Gly Ala Glu Tyr Ile Ala Arg Arg Cys Lys Glu Val Gly Val Leu Ala  
 260 265 270  
 15 Gly Arg Ser Pro Thr Thr Ile Ala Ala Thr Val Ile Tyr Met Ala Ser  
 275 280 285  
 Leu Val Phe Gly Phe Asp Leu Pro Pro Ser Lys Ile Ser Asp Lys Thr  
 290 295 300  
 20 Gly Val Ser Asp Gly Thr Ile Lys Thr Ser Tyr Lys Tyr Met Tyr Glu  
 305 310 315 320  
 Glu Lys Glu Gln Leu Ile Asp Pro Ser Trp Ile Glu Ser Gly Lys Val  
 325 330 335  
 Lys Leu Glu Lys Ile Pro Lys Asn  
 340

## (2) INFORMATION FOR SEQ ID NO:3:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 657 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 40 (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAAGTCAA	TAGAGGAAGA	TGAAAAAAT	AAAGCCGAAG	ATTTGGATAT	TATAAAAAAC	60
GAAGATATTG	ATGAACCTAA	ACAAGAAGAT	ACCACTGATA	GTAATGGTGG	TGGAGGTATT	120
GGTATAGTGC	CCACATTACA	AAATATTCTT	GCTACGGTGA	ATCTTGATTG	TCGACTTGAT	180
AAAACAATTG	CTTTACATGC	TAGAAATGCC	GAATATAATC	CAAAACGTTT	TGCTGCGGTG	240
ATTATGAGAA	TTAGAGATCC	AAAAACTACG	GCATTAATCT	TTGCTTCGGG	GAAAATGGTT	300
GTGACTGGGG	CTAAATCCGA	AGACGATTCC	AAGTTGGCTT	CAAGAAAGTA	TGCTAGAATC	360
ATTCAAAAGT	TGGGGTTCAA	TGCTAAATTT	TGTGATTTTA	AAATTCAAAA	TATAGTGGGG	420
TCAACAGATG	TTAAGTTTGC	TATTAGATTA	GAAGGCTTAG	CTTTTGCTCA	TGGTACTTTT	480
TCTTCATATG	AACCAGAAAT	ATTTCTGGG	TTAATTTATA	GAATGGTGAA	ACCAAAAATT	540
GTTTTACTTA	TATTTGTTTC	TGGGAAAATT	GTTTTGACGG	GTGCCAAAAA	GACAGAAGAA	600
ATTTATGATG	CATTTGAACT	GATTTATCCG	GTTTTAAATG	AATTTCTGTA	AAATTGA	657

## (2) INFORMATION FOR SEQ ID NO:4:

60 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1095 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY:

(ii) MOLECULE TYPE:  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	TAAGCTTGTA	TTACTAAGCA	TATTATGTCG	CCATCAACAT	CTACGGCAGT	ACAGGAGTAT	60
10	ATTGGACCAA	ACTTGAATGT	TACATTAACA	TGTCCTGAGT	GTAAGATATT	TCCACCTGAT	120
	TTGGTAGAGA	GGTTCAGCGA	AGGTGACATT	GTCTGTGGCA	GTTGTGGGCT	AGTATTGAGT	180
	GATCGTGTTG	TGGATACGAG	ATCAGAATGG	AGAACTTTCA	GTAACGATGA	CCAAAATGGT	240
	GATGATCCTT	CTCGTGTTGG	TGATGCAGGT	AACCCTTTAT	TAGACACAGA	GGACTTGTCC	300
	ACAATGATTT	CTTATGCTCC	TGATACTACC	AAAGCAGGAA	GAGAGTTAAG	CCGAGCCCAA	360
15	TCTAAATCTC	TAGTCGATAA	AAAAGACAAT	GCATTGGCTG	CAGCATATAT	CAAGATTCTT	420
	CAAAATGTGCG	ATGGTTATCA	ATTGCCTAAA	ATAGTTCTGG	ATGGGGCCAA	GGAAGTCTAC	480
	AAAATGGTTT	ATGACGAGAA	ACCATTGCGA	GGAAAATCAC	AAGAGAGTAT	CATGGCAGCT	540
	TCTATCTTTA	TTGGTTGCAG	AAAGGCCAAT	GTTGCTCGTT	CATTCAAAGA	GATATGGGCA	600
	AAGACTAATG	TACCTCGTAA	GGAAATTGGT	AAAGTGTTCA	AGATCATGGA	CAAGATCATT	660
20	CGTGAAAAGA	ATGCAGCCAA	CCCTAATGCT	GCATATTACG	GTCAAGACAG	CATTCAAACC	720
	ACCCAAACTT	CGGCCGAGGA	TTTGATTAGA	AGATTCTGTT	CTCACTTGGG	TGTTAACACA	780
	CAAGTTACAA	ATGGTGCGGA	ATACATAGCC	AGAAGATGTA	AGGAAGTCGG	GGTTTTAGCA	840
	GGTAGATCGC	CAACTACAAT	TGCTGCAACT	GTAATTTACA	TGGCTTCACT	AGTGTTTGGA	900
	TTTGACTTAC	CTCCATCCAA	GATATCTGAT	AAAACTGGTG	TCAGTGATGG	TACTATCAAA	960
25	ACTTCATACA	AGTACATGTA	CGAGGAGAAA	GAACAATTGA	TTGATCCATC	TTGGATAGAA	1020
	AGTGGTAAAG	TAAAATTGGA	AAAAATACCA	AAAAACTAAT	ACAGCGGAGT	CGCCACTGTT	1080
	AATCCTTTAC	CCTCT					1095

CLAIMS

1                   1.     A recombinant nucleic acid comprising a nucleic acid sequence  
2     encoding Candida albicans TBP.

1                   2.     A vector comprising a nucleic acid sequence encoding Candida  
2     albicans TBP.

1                   3.     A transformed host cell containing a nucleic acid sequence encoding  
2     Candida albicans TBP.

1                   4.     A recombinant polypeptide comprising Candida albicans TBP.

1                   5.     A fragment of Candida albicans TBP, said fragment being  
2     characterized in that it inhibits the biological activity of Candida albicans TBP in  
3     transcription initiation.

1                   6.     A fragment of Candida albicans TBP, said fragment being  
2     characterized in that it prevents the growth of Candida albicans.

1                   7.     A method for producing recombinant Candida albicans TBP,  
2     comprising culturing the host cell of claim 3 under conditions sufficient to permit  
3     expression of the nucleic acid encoding Candida albicans TBP, and isolating said Candida  
4     albicans TBP.

1                   8.     A screening method for identifying an inhibitor of Candida albicans  
2     growth, comprising detecting inhibition of mRNA transcription in an *in vitro* transcription  
3     assay comprising a DNA template, RNA polymerase II, recombinant Candida albicans  
4     TBP, and a candidate inhibitor, wherein production of an mRNA transcript from said  
5     DNA template occurs in the absence of said candidate inhibitor.

1                   9.     A screening method for identifying an inhibitor of Candida albicans  
2 growth, comprising detecting in the presence of a candidate inhibitor inhibition of  
3 formation of a complex comprising a DNA template and recombinant Candida albicans  
4 TBP, wherein in the absence of said candidate inhibitor, formation of said complex  
5 occurs.

1                   10.    A screening method for identifying an inhibitor of Candida albicans  
2 growth, comprising detecting in the presence of a candidate inhibitor inhibition of  
3 formation of a complex comprising Candida albicans TFIIB and Candida albicans TBP,  
4 wherein in the absence of said candidate inhibitor formation of said complex occurs.

1                   11.    A screening method for identifying an inhibitor of Candida albicans  
2 growth, comprising detecting in the presence of a candidate inhibitor inhibition of  
3 formation of a complex comprising RNA polymerase II, Candida albicans TBP, and  
4 Candida albicans TFIIB, wherein in the absence of said candidate inhibitor formation of  
5 said complex occurs.

1                   12.    The screening method of claim 8, 9, 10 or 11, wherein said  
2 detecting is performed in the presence of a plurality of candidate inhibitors such that said  
3 inhibition is indicative of inhibition by a said candidate inhibitor of said plurality.

1                   13.    The screening method of claim 8, 9, 10, or 11, wherein multiple  
2 detecting steps are performed simultaneously using a plurality of candidate inhibitors,  
3 wherein detection of inhibition by any one candidate inhibitor is detectable independently  
4 of said plurality.

1                   14.    A method of preventing Candida albicans growth in culture,  
2 comprising contacting said culture with an inhibitor that selectively inhibits the biological  
3 activity of Candida albicans TBP.

1                   15.    A method of preventing Candida albicans growth in a mammal,  
2 comprising administering to said mammal a therapeutically effective amount of an  
3 inhibitor that inhibits the biological activity of Candida albicans TBP.

397/1  
 ATG AAG TCA ATA GAG GAA GAT GAA AAA AAT AAA GCC GAA GAT TTG GAT ATT ACA AAA AAC  
 M K S I E E D E K N F A E D L D I I K K  
 457/21  
 GAA GAC ATT GAT GAA COT AAA CAA GAA GAT ACC ACT GAT AGT AAT GGT GGT CGA GGT ATT  
 I D I D E P K Q B D T C D S N G G G G I  
 517/41  
 GGT ATA GTG CCC ACA TTA CAA AAT ATT GTT GGT ACG GTG AAT GTT GAT TGT CGA GTT GAT  
 G I V F T L Q N I V A T V N L D C E L E  
 577/61  
 GTT AAA ACA ATT GGT TTA CAT GGT AGA AAT CCC GAA TAT AAC CCA AAA COT TTT GGT GCG  
 L K T I A L M A R N A E Y N P E R F A A  
 637/81  
 GTG ATT ATC ACA ATT ACA GAT CGA AAA ACT ACG GCA TTA ATC TTT GGT TCG GCG AAA ATG  
 V I M R I R D P R T T E L I F L S G K M  
 697/101  
 GTT GTG ATT GCG GGT AAA TCC GAA GAC GAT TCC AAG TTG GGT TCA AGA AAG CAT GGT ACA  
 V V T G A K S E D D S K L A S R K Y A R  
 757/121  
 ATC ATT CAA AAG TTG GCG TTC AAT GGT AAA TTT TGT GAT TTT AAA ATT CAA AAT ATA GTG  
 I I Q K L G F N A K F C D P M I Q N I V  
 817/141  
 GCG TCA ACA GAT GTT AAG TTT GGT ATT AGA TTA GAA GCG TTA GGT TTT GGT CAT GGT ATT  
 G S T D V K P A I R L E G L A P A E G T  
 877/161  
 TTC TTT TCA TAT GAA CCA GAA TTA TTT COT GCG TTA ATT TAC AGA TTG GTG AAA CCA AAA  
 T S S Y E P E L P P G L I Y R M V K P R  
 937/181  
 ATT GTT TTA GTT AAT TTT GTT TTT GCG AAA ATT GTT TTG ACC GGT GGT AAA AAC ACA GAA  
 I V L L I F V S G K I V L T G A K K R E  
 997/201  
 GAA ATT TAT GAT GCA TTT GAA GTG ATT TAT CCG GTT TTA AAT GAA TTT COT AAA AAT TGA  
 E I Y D A P I X I Y P V L N E F R K N \*

X  
 S

FIG 1

2/2

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1 TAAGCTTGTATTACTAAGCATATT ATG TCG CCA TCA ACA TCT ACC GCA GTA CAG GAG TAT ATT GGA 66
1 M S P S T S T A V Q E Y I G 14

67 CCA AAC TTG AAT GTT ACA TTA ACA TGT COT GAG TOT AAG ATA TTT CCA COT GAT TTG GTA 126
15 P N L N V T L T C P E C K I F P P D L V 34

127 GAG AGG TTC AGC GAA GGT GAC ATT GTC TOT GGC AGT TOT GGC CTA GTA TTG AGT GAT COT 186
35 E R F S E G D I V C G S C G L V L S D R 54

187 GTT GTG GAT ACC AGA TCA GAA TGG AGA ACT TTC AGT AAC GAT GAC CAA AAT GGT GAT GAT 246
55 V V D T R S E W R T F S N D D Q N G D D 74

247 COT TCT COT GTT GGT GAT GCA COT AAC COT TTA TTA GAC ACA GAG GAC TTG TCC ACA ATG 306
75 P S R V G D A C N P L L D T E D L S T M 94

307 ATT TCT TAT GCT COT GAT AGT ACC AAA GCA GGA AGA GAG TTA ACC CGA GCC CAA TCT AAA 366
95 I S Y A P D S T K A C R E L S R A Q S K 114

367 TCT CTA GTC GAT AAA AAA GAC AAT GCA TTG GCT GCA GCA TAT ATC AAG ATT TCT CAA ATG 426
115 S L V D R K K D N A L A A A Y I K I S Q M 134

427 TGC GAT GGT TAT CAA TTG COT AAA ATA GTT CTG GAT GGG GCC AAG GAA GTC TAC AAA ATG 486
135 C D G Y Q L P K I V SS D G A K E V Y K M 154

487 GTT TAT GAC GAG AAA CCA TTG CGA GGA AAA TCA CAA GAG AGT ATC ATG GCA GCT TCT ATC 546
155 V Y D E K P L R G K S Q E S I M A A S I 174

547 TTT ATT GGT TGC AGA AAG GCC AAT GTT GCT COT TCA TTC AAA GAG ATA TGC GCA AAG ACT 606
175 F I G C R K A N V A R S F K E I W A K T 194

607 AAT GTA COT COT AAG GAA ATT GGT AAA GTG TTC AAG ATC ATG GAC AAG ATC ATT COT GAA 666
195 N V P R K E I G K V F K I M D K I I R E 214

667 AAG AAT GCA GCC AAC COT AAT GGT GCA TAT TAC GGT CAA GAC AGC ATT CAA ACC ACC CAA 726
215 F N A A N P N A A Y Y C Q D S I Q T T Q 234

727 ACT TCG GCC GAG GAT TTG ATT AGA AGA TTC TOT TCT CAC TTG GGT GTT AAC ACA CAA GTT 786
235 T S A E D L I R R F C S H L G V N T Q V 254

787 ACA AAT GGT GCG GAA TAC ATA GCC AGA AGA TOT AAG GAA GTC GGG GTT TTA GCA GGT AGA 846
255 T N G A E Y I A R R C K E V G V L A G R 274

847 TCG CCA ACT ACA ATT GCT GCA ACT GTA ATT TAC ATG GCT TCA CTA GTG TTT GGA TTT GAC 906
275 S P T T I A A T V I Y M A S L V F G F D 294

907 TTA COT CCA TCC AAG ATA TCT GAT AAA ACT GGT GTC AGT GAT GGT ACT ATC AAA ACT TCA 966
295 L P P S K I S D K T C V S D G T I K T S 314

967 TAC AAG TAC ATG TAC GAG GAG AAA GAA CAA TTG ATT GAT CCA TCT TGG ATA GAA AGT GGT 1026
315 Y X Y M Y E E K E Q L I D P S W I E S G 334

1027 AAA GTA AAA TTG GAA AAA ATA CCA AAA AAC TAA TACAGCGGAGTCGCCACTGTTAATCCTTTACCCCTCT 1095
335 K V K L E K I P K N 345

```

FIG. 2

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/06170

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) Please See Extra Sheet.

US CL Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U S : 435/6, 7.8, 29, 69.1, 70.1, 71.1, 243, 320.1, 325, 530/350, 536/23.74

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	PETERSON et al. Transcription factor based therapeutics: drugs of the future?. Trends in Biotechnology. January 1993. Vol. 11. pages 11-18, see entire document.	1-15
A	BURATOWSKI et al. Mechanisms of Gene Activation. Science. 15 December 1995. Vol. 270. pages 1773-1774, see entire document.	1-15
A	POON et al. Immunopurification of Yeast TATA-binding Protein and Associated Factors. The Journal of Biological Chemistry. 25 July 1993. Vol. 268. No. 21. pages 15325-15328, see entire document.	1-15

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 MAY 1997

Date of mailing of the international search report

09 JUL 1997

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Authorized officer

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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)\*

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US97/06170

## C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	POON et al. Yeast Taf170 is Encoded by MOT1 and Exists in a TATA Box-binding Protein (TBP)-TBP-associated Factor Complex Distinct from Transcription Factor IID. The Journal of Biological Chemistry. 16 September 1994. Vol. 269. No. 37. pages 23135-23140. see entire document.	1-15
A, P	US 5,534,410 A (TJIAN et al) 09 July 1996, see entire document.	1-15
A, P	US 5,569,588 A (ASHBY et al) 29 October 1996, see entire document.	1-15

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/06170

## A. CLASSIFICATION OF SUBJECT MATTER

IPC (6)

C07K 14/40; C12N 1/00, 5/10, 15/31, 15/63; C12P 21/04, 21/06, C12Q 1/02, 1/68, G01N 33/53

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL

435/6, 7.8, 29, 69.1, 70.1, 71.1, 243, 320.1, 325; 530/350; 536/23.74

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used)

### APS, DIALOG

search terms: TBP, tata binding protein, tata box binding protein, transcription factor, candida albicans, tfid, screen?, assay?, transcri?, inhibit?, detect?, identify?, compound?, drug?, candidate, pharmaceutical?, rna polymerase ii, rna polii, complex?, administer?, treat?, tfiib





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> C07K 14/40, C12N 1/00, 5/10, 15/31, 15/63, C12P 21/04, 21/06, C12Q 1/02, 1/68, G01N 33/53	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/36925</b> <b>(43) International Publication Date:</b> 9 October 1997 (09.10.97)
<b>(21) International Application Number:</b> PCT/US97/06170 <b>(22) International Filing Date:</b> 31 March 1997 (31.03.97)  <b>(30) Priority Data:</b> 626,309 1 April 1996 (01.04.96) US  <b>(71) Applicants:</b> SCRIPTGEN PHARMACEUTICALS, INC. [US/US]; 200 Boston Avenue, Medford, MA 02155 (US). PRESIDENT & FELLOWS OF HARVARD COLLEGE [US/US]; 124 Mt. Auburn Street, Cambridge, MA 02138 (US).  <b>(72) Inventors:</b> BURATOWSKI, Stephen; 706 Webster Street, Needham, MA 02912 (US). BURATOWSKI, Robin; 706 Webster Street, Needham, MA 02912 (US). WOBBE, C., Richard; 57 Spring Street, Lexington, MA 02173 (US). BRADLEY, John; 25 Parkman Street #1, Brookline, MA 02147 (US).  <b>(74) Agents:</b> LUDWIG, S., Peter et al.; Darby & Darby P.C., 805 Third Avenue, New York, NY 10022 (US).		<b>(81) Designated States:</b> CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> CANDIDA ALBICANS TATA-BINDING PROTEIN, NUCLEIC ACID AND ASSAYS  <b>(57) Abstract</b>  The invention encompasses a novel transcription factor from <i>Candida albicans</i> , TBP, a nucleic acid sequence encoding TBP, and methods of screening for inhibitors of <i>Candida albicans</i> growth by targeting TBP.		

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CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
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CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
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## CANDIDA ALBICANS TATA-BINDING PROTEIN, NUCLEIC ACID AND ASSAYS

ABSTRACT OF THE DISCLOSURE

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The invention encompasses a novel transcription factor from *Candida albicans*, TBP, a nucleic acid sequence encoding TBP, and methods of screening for inhibitors of *Candida albicans* growth by targeting TBP.

The invention relates in general to transcription factors and to methods for screening for antifungal agents.

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The invention was made in part using government funds, NIH grant no. GM46498, and therefore the U.S. government has certain rights in the invention.

BACKGROUND OF THE INVENTION

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The yeast *Candida albicans* (*C. albicans*) is one of the most pervasive fungal pathogens in humans. It has the capacity to opportunistically infect a diverse spectrum of compromised hosts, and to invade many diverse tissues in the human body. It can in many instances evade antibiotic treatment and the immune system. Although *Candida albicans* is a member of the normal flora of the mucous membranes in the respiratory, gastrointestinal, and female genital tracts, in such locations, it may gain dominance and be associated with pathologic conditions. Sometimes it produces progressive systemic disease in debilitated or immunosuppressed patients, particularly if cell-mediated immunity is impaired. Sepsis may occur in patients with compromised cellular immunity, e.g., those undergoing cancer chemotherapy or those with lymphoma, AIDS, or other conditions. *Candida* may produce bloodstream invasion,

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thrombophlebitis, endocarditis, or infection of the eyes and virtually any organ or tissue when introduced intravenously, e.g., via tubing, needles, narcotics abuse, etc.

*Candida albicans* has been shown to be diploid with balanced lethals, and therefore probably does not go through a sexual phase or meiotic cycle. This yeast  
5 appears to be able to spontaneously and reversibly switch at high frequency between at least seven general phenotypes. Switching has been shown to occur not only in standard laboratory strains, but also in strains isolated from the mouths of healthy individuals.

Nystatin, ketoconazole, and amphotericin B are drugs which have been used to treat oral and systemic *Candida* infections. However, orally administered nystatin is  
10 limited to treatment within the gut and is not applicable to systemic treatment. Some systemic infections are susceptible to treatment with ketoconazole or amphotericin B, but these drugs may not be effective in such treatment unless combined with additional drugs. Amphotericin B has a relatively narrow therapeutic index and numerous undesirable side effects and toxicities occur even at therapeutic concentrations. While ketoconazole and  
15 other azole antifungals exhibit significantly lower toxicity, their mechanism of action, inactivation of cytochrome P<sub>450</sub> prosthetic group in certain enzymes, some of which are found in humans, precludes use in patients that are simultaneously receiving other drugs that are metabolized by the body's cytochrome P<sub>450</sub> enzymes. In addition, resistance to these compounds is emerging and may pose a serious problem in the future.

20 There is a need in the art for an effective treatment of opportunistic infections caused by *Candida albicans*. Therefore, one object of the invention is to provide screening assays for identifying potential inhibitors of *Candida albicans* growth. Another object of the invention is to provide screening assays and to identify potential inhibitors of *Candida albicans* growth that are based on inhibition of transcription in this  
25 organism.

Synthesis of mRNA in eukaryotes requires RNA polymerase II and accessory transcription factors, some of which are general and act at most, if not all, as promoters, and others of which confer specificity and control. Five general factors, a, b, d, e, and g, have been purified to homogeneity from the yeast *S. cerevisiae*, and have  
30 been identified as counterparts of human or rat factors, TFIIE, TFIIH, TFIID, TFIIB and TFIIIF, respectively. These factors assemble at a promoter in a complex with RNA polymerase II to initiate transcription. Binding studies have shown that the order of assembly of the initiation complex on promoter DNA begins with factor d (TFIID), is

followed by factor e (TFIIB), and then by polymerase and the remaining factors. Factors b (TFIIH), e (TFIIB) and g (TFIIF), however, bind directly to polymerase II, and as many as four of the five factors may assemble with the polymerase in a holoenzyme before promoter binding. The functional significance of interactions revealed by binding studies is not clear in that only a few percent of initiation complexes may give rise to transcripts.

Many aspects of transcription by RNA polymerase II are conserved between yeast and higher eukaryotes. For example, there is extensive amino acid sequence similarity among the largest subunits of the yeast, *Drosophila* and mammalian polymerases. Other components of the transcription apparatus, such as TATA-binding and enhancer binding factors, are in some instances interchangeable between yeast and mammalian *in vitro* binding or transcription systems. There are, nonetheless, significant differences between the two systems. TATA elements are located from 40 to 120 or more base pairs upstream of the initiation site of an *S. cerevisiae* promoter, and where these elements occur, they are required for gene expression. The fact that *C. albicans* genes function in *S. cerevisiae* suggests that it also uses the 40 to 120 base pair spacing between the TATA element and initiation site. In contrast, mammalian (as well as *S. pombe*) TATA elements and transcription start sites are only 25 to 30 bp apart, and deletion of a TATA element does not always reduce the frequency of transcription initiation, although it may alter the initiation site. There are also varying degrees of homology between transcription factor sequences from yeast and mammalian sources. Some of the multisubunit factors, such as RNA polymerase II, TFIIF, and TFIID, contain different numbers of subunits in humans and yeast. The molecular weights of corresponding polypeptides differ between humans and yeast, with sequences being found in a given yeast factor not being found in its human counterpart and vice versa.

TATA-binding protein (TBP) is the central initiation factor for transcription by all three nuclear RNA polymerases, and is highly conserved throughout the eukaryotic kingdom. The 180 amino acid carboxy-terminal core domain is sufficient for TATA element binding, for all essential functions in *S. cerevisiae*, and is 80% identical between *S. cerevisiae* and humans. *In vitro*, yeast and human TBPs can functionally replace one another in terms of basal RNA polymerase II transcription, and they display nearly identical DNA sequence requirements for TATA elements. However, TBP exhibits species-specific behavior *in vivo*. For example, human and yeast TBP's are not species

interchangeable in supporting cell growth (Gill and Tjian, Cell 65:333-340, (1991); Cormack et al., Cell 65:341-348 (1991)). Human and *S. cerevisiae* TFIIB's have 50-60% amino acid sequence identity, and also are not species interchangeable in supporting cell growth.

5           Operative substitution of the same transcription factor in transcription systems of different yeast species is not predictable. This is true despite a high degree of amino acid sequence identity among some transcription factors from different yeast species. For example, the ability of a given transcription factor to support efficient and accurate transcription in a heterologous yeast species is not predictable. Li et al. (1994, 10 Science 263:805) tested the interchangeability of *S. cerevisiae* and *S. pombe* transcription factors *in vitro*, and report that many *S. cerevisiae* components cannot substitute individually for *S. pombe* RNA transcription factors a, e, or polymerase II, but some combinations of these components were effective. In one instance, active transcription could not be reconstituted when *S. cerevisiae*-derived TFIIB was the sole substitution into 15 a TFIIB-depleted set of factors from *S. pombe*. A TFIIB-RNA polymerase II combination from *S. cerevisiae* was able to substitute, indicating that the functional interaction of these two components is not only important, but also that the activity may be dependent on species-specific determinants that cannot be complemented by either component derived from a different organism. The unpredictability in making substitutions of a given factor 20 among different yeast species is also evident in that such substitutions are not reciprocal; that is, substitutions of *S. pombe* fractions into an *S. cerevisiae* transcription system are less effective than the reverse substitutions (Li et al., supra).

          The yeast *Candida albicans* differs from most yeast strains in that it does not use the same genetic code that most organisms, whether mammalian or yeast, utilize. 25 Santos et al. (1995, Nucleic Acids Research, 23:1481) report that the codon CUG, which in the universal code is read as a leucine, is decoded as a serine in *Candida*. Therefore, any CUG codon that is decoded in *Candida albicans* as a serine, would be decoded as a leucine in the transformed *S. cerevisiae*. Any gene containing a CUG codon would therefore be translated as different amino acid sequences in *Candida albicans* and *S.* 30 *cerevisiae*. Such mistranslation may produce an inactive protein, since the amino acids serine and leucine have markedly different chemical properties and serine is known to be an essential residue in the active site of some enzymes. Replacement of leucine by serine

at CUG encoded residues is a serious problem in the use of many reporter systems (e.g.  $\beta$ -galactosidase, Chloramphenicol acetyltransferase, Flux) in Candida albicans. Previous experiments have shown that translation by *Candida* of CUG as serine instead of leucine often resulted in the production of inactive reporter proteins.

5 Another object of the invention is to provide an assay for screening for selective inhibition of Candida albicans growth and/or viability.

Yet another object of the invention is to provide a molecular target for inhibition of Candida albicans transcription or transcription initiation.

## 10 SUMMARY OF THE INVENTION

The invention encompasses a recombinant nucleic acid comprising a nucleic acid sequence encoding Candida albicans TBP.

The invention also encompasses a vector comprising a nucleic acid sequence encoding Candida albicans TBP, and a transformed host cell containing a nucleic acid  
15 sequence encoding Candida albicans TBP.

The invention also encompasses a method for producing recombinant Candida albicans TBP, comprising culturing a host cell transformed with a nucleic acid encoding Candida albicans TBP under conditions sufficient to permit expression of the nucleic acid encoding Candida albicans TBP, and isolating Candida albicans TBP.

20 The invention also encompasses a screening method for identifying an inhibitor of Candida albicans growth, comprising detecting inhibition of mRNA transcription in an *in vitro* transcription assay comprising a DNA template, RNA polymerase II, recombinant Candida albicans TBP, and a candidate inhibitor, wherein production of an mRNA transcript complementary to the DNA template occurs in the  
25 absence if the candidate inhibitor.

The invention also encompasses a screening method for identifying an inhibitor of Candida albicans growth, comprising detecting in the presence of a candidate inhibitor inhibition of formation of a complex comprising a DNA template and recombinant Candida albicans TBP, wherein in the absence of the candidate inhibitor,  
30 formation of the complex occurs. The method also may be performed in the presence of additional factors, such as TFIIB, RNA polymerase II and TFIIF.

The invention also encompasses a screening method for identifying an

inhibitor of Candida albicans growth, comprising detecting in the presence of a candidate inhibitor, inhibition of formation of a complex comprising Candida albicans TFIIB and Candida albicans TBP, wherein in the absence of the candidate inhibitor formation of the complex occurs. Preferably, the complex will include a DNA template.

5 The invention also encompasses a screening method for identifying an inhibitor of Candida albicans growth, comprising detecting in the presence of a candidate inhibitor inhibition of formation of a complex comprising RNA polymerase II, Candida albicans TBP, and Candida albicans TFIIB, wherein in the absence of the candidate inhibitor formation of the complex occurs. Preferably, the complex will include a DNA  
10 template and the RNA polymerase II from *C. albicans*.

In the above-described screening methods, detection may be performed in the presence of a plurality of candidate inhibitors. In screening methods of the invention which involve screening of a plurality of candidate inhibitors, the plurality of inhibitors may be screened together in a single assay or individually using multiple simultaneous  
15 individual detecting steps.

The invention also encompasses a method of preventing Candida albicans growth in culture, comprising contacting the culture with an inhibitor that selectively inhibits the biological activity of Candida albicans TBP.

The invention also encompasses a method of preventing Candida albicans  
20 growth in a mammal, comprising administering to a mammal a therapeutically effective amount of an inhibitor that inhibits the biological activity of Candida albicans TBP.

As used herein, "inhibition" refers to a reduction in the parameter being measured, whether it be Candida albicans growth or viability, Candida albicans TBP-mediated transcription, or formation of a Candida albicans TBP transcription complex.  
25 The amount of such reduction is measured relative to a standard (control). Because of the multiple interactions of Candida albicans TBP in transcription initiation, the target product for detection varies with respect to the particular screening assay employed. Three preferred detection products presented in this disclosure are; a) newly transcribed mRNA, b) a DNA-TBP complex, and c) a TBP-TFIIB-RNA polymerase II complex.  
30 "Reduction" is defined herein as a decrease of at least 25% relative to a control, preferably of at least 50%, and most preferably of at least 75%.

As used herein, "growth" refers to the normal growth pattern of Candida

albicans, i.e., to a cell doubling time of 60 - 90 minutes. "Viability" refers to the ability of Candida albicans to survive in culture for 48 hours.

"Biological activity" refers to the ability of TBP to form a transcription complex with a DNA template or other proteins of the transcription complex, or to  
5 interact with other transcription components so as to permit initiation of transcription.

"DNA template" refers to double stranded DNA and, where indicated by the particular binding assay to single stranded DNA, at least 10 nucleotides in length, that may be negatively supercoiled if double-stranded, possesses a promoter region, and contains a yeast TATA consensus region. DNA templates useful herein preferably will  
10 contain a TATA sequence that is located from 40 to 120 or more base pairs upstream of the initiation site (distance measured from the first T of the TATA element to the 5'-most initiation site). An especially efficient DNA template for use in methods of the invention involving transcription is devoid of guanosine residues, and therefore a "G-minus" or "G-less" cassette is preferred.

15 "mRNA transcript" refers to a full-length transcript as well as to truncated transcripts, oligonucleotide transcripts and dinucleotide RNAs.

"Formation of a complex" refers to the binding of TBP to other transcription factors (i.e., protein-protein binding) as well as to binding of TBP to a DNA template; such binding will, of course, be a non-covalent association.

20 Other features and advantages of the invention will be apparent from the description, preferred embodiments thereof, the drawings, and from the claims.

### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 presents the nucleotide and amino acid sequences of the Candida  
25 albicans transcription factor TBP.

Fig. 2 presents nucleotide and amino acid sequence of the Candida albicans transcription factor TFIIB.

### DESCRIPTION

30 The invention is based on the discovery of a novel protein, Candida albicans TBP, and on the isolation of recombinant DNA encoding Candida albicans transcription factor TBP. Because TBP is essential for viability of the cell, a compound that blocks the biological activity of the protein is expected to have fungicidal properties.

Therefore, the invention is also based on the development of assays for screening from inhibitors of TBP.

Isolation and Characterization of the *Candida albicans* TBP Gene

Given the unpredictability with respect to operative substitutions of a given transcription factor among different yeast strains, one cannot assume that strategies for cloning of the gene encoding a given transcription factor which are based on factor function, such as genetic complementation, will work. Other cloning strategies, which do not require functional complementation, such as those based on homology at the nucleic acid level, may be utilized in an attempt to circumvent a requirement for factor function. For example, Southern hybridization of specific sequences to a library carried in *E. coli* and PCR amplification of potentially highly homologous regions of a gene are two strategies that have been successfully used to clone homologous genes from different organisms.

The approach used to clone the *Candida albicans* homolog of TBP involved genetic complementation of mutant *S. cerevisiae* strains. A library of *Candida albicans* genomic sequences was introduced into a strain of *S. cerevisiae* that contained a mutated TBP gene (*spt15*). This mutant strain was capable of growth at 30° C, but was non-viable at 37° C, due to a temperature sensitive mutation in the TBP gene. Following transformation of the library into the strain, the cells were grown at 37° C, and the colonies which grew at this non-permissive temperature were further studied as potentially carrying a *Candida albicans* homolog of the defective gene. This approach will only work if a *Candida albicans* homolog is able to substitute functionally *in vivo* for the defective gene.

After candidate clones were isolated by growth at the nonpermissive temperature, the library plasmid DNA was recovered from the cell and retested to confirm that the *C. albicans* sequences on the plasmid were substituting for the *S. cerevisiae* gene. Subclones of the *C. albicans* sequences were constructed by standard cloning methods, and the minimal *Candida* DNA sequences that substituted were sequenced using standard methods.

The nucleotide sequence encoding *Candida albicans* TBP and the predicted amino acid sequence of the encoded protein are presented in Fig. 1 (SEQ ID NOS: 1 and 2). The nucleotide sequence encoding *Candida albicans* TFIIB and the predicted amino

acid sequence of the encoded protein are presented in Fig. 2 (SEQ ID NOS: 3 and 4).

#### Methods For Screening Potential Inhibitors of *Candida albicans* Growth and/or Viability

Because TBP initiation factor is essential for transcription initiation, the recombinant *Candida albicans* TBP gene and recombinant protein encoded by this gene may be utilized in screening assays for inhibitors of *Candida albicans* growth and viability. The screening assays of this invention detect inhibition of the *Candida albicans* TBP-mediated component of transcription initiation, either by measuring inhibition of transcription, transcription initiation, or initiation complex formation, or by assaying formation of a protein/DNA or a protein/protein complex.

#### EXAMPLE 1

##### Screening for Inhibitors of Transcription

###### a) Transcription Assay Components.

An *in vitro* transcription assay consisting of the minimal components necessary to synthesize an mRNA transcript from a DNA template can be used to screen for inhibition of mRNA production. The elements of such an assay consist of; a) a DNA template, b) RNA polymerase II, c) recombinant *Candida albicans* TBP, and d) a TFIIB which is preferably *Candida albicans* TFIIB. In order to increase the efficiency of transcription, additional components of the transcription complex may be included, as desired; e.g., TFIIE, TFIIF, TFIIH, etc.

Parvin and Sharp (*Cell* 73, 533-540, 1993) have reconstituted gene transcription *in vitro* with a minimal reaction containing a DNA template, RNA polymerase II, TFIIB, and TBP. For efficient transcription under minimal conditions, the DNA template (a) is supercoiled, and (b) possesses a promoter region containing a TATA consensus region. Additionally, Lue et al. (*Science* 246, 661-664, 1989) have determined that transcription may be detected most efficiently with a DNA template devoid of guanosine residues (a G-minus or G-less cassette). Promoter dependence is demonstrated by the loss of signal when a plasmid lacking promoter sequences is utilized as a template. Correct initiation is demonstrated by the production of a band with a mobility consistent with the size of the expected product on denaturing polyacrylamide electrophoresis gels.

As stated above, *Candida albicans* TBP forms a transcription initiation complex with RNA polymerase II. Therefore, it is desired that an *in vitro* transcription

assay according to the invention contain RNA polymerase II. Although it is possible to perform an inhibitor screening assay using RNA polymerase II from a yeast strain other than *Candida albicans*, e.g., *S. cerevisiae*, it is most desirable to use a homologous assay in which the transcription complex components are from *Candida albicans*.

5 A method for *S. cerevisiae* RNA polII purification is described in Edwards et al. (*Proc. Natl. Acad. Sci. USA* 87: 2122-2126 (1990)). Alternatively, highly purified RNA polymerase II from *Candida albicans* was provided as follows.

RNA polymerase II activity was measured in reactions containing 50 mM Tris-Cl, pH 7.9 (4° C), 50 mM (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 2.5 mM MnCl<sub>2</sub>, 0.1 mM EDTA, 5 mM  
10 DTT, 100 µg/ml BSA, 0.6 mM ATP, CTP and GTP, 25 µM UTP (2.5 µCi) [ $\alpha$ -<sup>32</sup>P] UTP and 100 µg/ml heat-denatured calf thymus DNA in a final volume of 50 µl. Reactions were incubated for 60 min. at 30° C and terminated by addition of 50 µl 15% (w/v) trichloroacetic acid. Acid-insoluble radioactivity was collected by filtration through glass fiber filters and quantified by liquid scintillation spectrophotometry. One unit of RNA  
15 polymerase activity catalyzes the incorporation of 1 pmol of UTP into acid-insoluble material in 60 min. under the conditions described above.

*Candida albicans* was obtained from the American Type Culture Collection (ATCC 10231) and cultured in YPD medium (Current Protocols in Molecular Biology, Vol. 2, 13, Suppl. 19 (1989)) at 30° C with vigorous agitation and aeration. All  
20 procedures were carried out at 4° C using 18 liter cultures. Cells were harvested by centrifugation (5000 rpm, 10 min., Sorvall H6000 rotor), washed once with ~ 1 l ice-cold deionized water and repelleted as above. The cell pellet (200-300 g wet weight) was thoroughly resuspended in a volume of Buffer A (50 mM Tris-HCl, pH 7.9, 4° C, 10% glycerol, 1 mM EDTA, 5 mM MgCl<sub>2</sub>, and protease inhibitor) containing 300 mM (NH<sub>4</sub>)<sub>2</sub>  
25 SO<sub>4</sub> equivalent to the packed volume of cells (determined by weight assuming a density of 1 g/ml cells). Resuspended cells were either processed immediately as described below or frozen by pipetting into liquid N<sub>2</sub> and stored at -80 C. Frozen cells were thawed on ice prior to proceeding. Following the addition of NP-40 to a final concentration of 0.1%, cells were disrupted by grinding with 1 ml acid-washed glass beads/ml cell  
30 suspension (Sigma, 400-625 µM) using 12 bursts of 30 sec. each in a Bead Beater (BioSpec). Glass beads were allowed to settle out and the supernatant was centrifuged at 30,000 x g for 40 min. Solid (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> was slowly added to a final concentration of 0.4 g/ml supernatant and the resulting precipitate was pelleted by centrifugation at 100,000

x g for 30 min. The pellet was resuspended with a volume of Buffer A sufficient to yield a conductivity equivalent to Buffer A containing 75 mM  $(\text{NH}_4)_2 \text{SO}_4$ .

Following centrifugation of the resuspension at 10,000 x g for 10 min, this supernatant (~ 1-1.5 mg protein/ml) was loaded onto a 300 ml DE-52 DEAE-cellulose column equilibrated with Buffer A containing 75 mM  $(\text{NH}_4)_2 \text{SO}_4$ . After washing with 5 column volumes Buffer A containing 75 mM  $(\text{NH}_4)_2 \text{SO}_4$ , and 5 column volumes Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ , RNA polymerase II was eluted with 5 column volumes Buffer A containing 0.4 M  $(\text{NH}_4)_2 \text{SO}_4$ . Fractions were collected containing the peak of protein, determined by absorbance at 280 nm and pooled. The pool was dialyzed against Buffer A containing 20% glycerol for 3 hr. at 4° C.

The 0.4 M  $(\text{NH}_4)_2 \text{SO}_4$  eluate from DEAE-cellulose (261 mg protein, 290 ml) was diluted with sufficient Buffer A to lower the conductivity to the equivalent of Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ , centrifuged at 10,000 x g for 10 min. and the supernatant was loaded at a flow rate of 30 ml/hr onto a 30 ml DEAE-cellulose column equilibrated with Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ . After washing with 3 column volumes of Buffer A containing 0.15 M  $(\text{NH}_4)_2 \text{SO}_4$ , the column was developed with a 200 ml linear gradient of 0.15 - 0.4 M  $(\text{NH}_4)_2 \text{SO}_4$  in Buffer A at a flow rate of 45 ml/hr. Fractions from the single peak of amanitin-sensitive RNA polymerase activity, eluting around 0.22 M  $(\text{NH}_4)_2 \text{SO}_4$ , were pooled (21.1 mg protein, 45 ml) and loaded directly onto a 5 ml Heparin agarose column equilibrated with Buffer A containing 0.2 M  $(\text{NH}_4)_2 \text{SO}_4$ . The column was washed with 3 column volumes of Buffer A containing 0.2 M  $(\text{NH}_4)_2 \text{SO}_4$  and developed with an 80 ml linear gradient of 0.2 - 0.6 M  $(\text{NH}_4)_2 \text{SO}_4$  in Buffer A. The active fractions, which eluted at approximately 0.42 M  $(\text{NH}_4)_2 \text{SO}_4$  were pooled (2.0 mg protein, 15 ml), frozen in 300  $\mu\text{l}$  aliquots in liquid  $\text{N}_2$ , and stored at -80° C where activity was stable for at least 6 months.

Purification of protein initiation factors used in the assay is accomplished by standard methods known in the art (e.g., phosphocellulose chromatography followed by gel filtration), as described in (*Nature* 346, 387-390 (1990)).

To screen for *Candida albicans* TBP-mediated transcription inhibition, a transcription assay is reconstituted using recombinant *Candida albicans* TBP. Supercoiled plasmid DNA containing the CYC1 promoter linked to the G-less cassette described by Lue *et al.* (*Science* 246, 661-664 (1989)), is purified by standard methods for purification of supercoiled circular DNA (Current Protocols in Molecular Biology,

Vol. 2, 13, Suppl. 19 (1989)). 10 - 100 ng of *Candida albicans* TFIIB, 10 - 100 ng of *Candida albicans* TBP, 10 - 100 ng *Candida albicans* RNA polymerase II and 1  $\mu$ g plasmid DNA are added to 50  $\mu$ l reaction mixtures containing 50 mM HEPES, pH 7.5, 10% glycerol, 90 mM potassium glutamate, 0.75% polyethylene glycol (molecular weight 3350), 10 mM magnesium acetate, 5 mM EGTA, 5 mM DTT, 0.4 mM ATP, 0.4 mM CTP, 10  $\mu$ M [ $\alpha$ - $^{32}$ P]UTP, 0.2 mM 3'-O-methyl-GTP, and containing or lacking a candidate inhibitor molecule. Reactions are incubated at 30 $^{\circ}$  C for 30 - 60 min. and RNA synthesis is detected as described below.

b) Detection of Transcribed RNA.

The detection of newly transcribed RNA is achieved by standard methods (Current Protocols in Molecular Biology, Vol. 1, 4.10, Suppl 24 (1989)). As one example, RNA synthesis can be detected as incorporation of a radioactively or fluorescently labeled nucleotide into higher molecular weight RNA products, determined by one of the following methods: 1) acid-insoluble labeled material quantitated by the appropriate method (e.g. scintillation counting for radioactive precursors, fluorometry for fluorescent precursors); 2) labeled reaction product that hybridizes to oligonucleotides complementary to the correctly initiated transcript (i.e., northern blot analysis); 3) the presence of a labeled band with the appropriate mobility detected by autoradiography, on denaturing polyacrylamide electrophoresis gels; 4) any other method that discriminates mononucleotides from polynucleotides, where polynucleotides are the desired RNA product. Such methods may utilize one or more well known techniques of molecular biology (Current Protocols in Molecular Biology, Vol. 2, 13, Suppl. 19 (1989)), for example; UV analysis; affinity systems (e.g., affinity chromatography, nitrocellulose filtration, biotin/streptavidin systems, immunoaffinity,) (Current Protocols in Molecular Biology, Vol. 2, 13, Suppl. 19 (1989)); and high performance liquid chromatography.

The inclusion of an inhibitor molecule that interferes with *Candida albicans* TBP biological activity inhibits transcription. In this assay inhibition is measured as a reduction in the amount of mRNA transcript produced relative to the amount of mRNA transcript produced in the absence of the inhibitor (the positive control). A decrease in amount of mRNA transcript is indicative of an inhibitor. The determination of effective levels of mRNA transcript inhibition is described below.

## EXAMPLE 2

### Screening for Inhibition of DNA-Protein Complex Formation

A DNA-protein binding assay consisting of the minimal components necessary to permit DNA-*Candida albicans* TBP binding to occur can be used to screen  
5 for inhibition of the formation of the DNA-*Candida albicans* TBP complex during transcription initiation. The essential elements of such an assay consist of; a) a DNA template, b) recombinant *Candida albicans* TBP, and optionally c) a candidate *Candida albicans* TBP inhibitor.

The inclusion of an inhibitor molecule that interferes with the interaction  
10 between the *Candida albicans* TBP and the DNA template inhibits transcription initiation. The inhibitor may interact directly with the *Candida albicans* TBP protein, and/or it may interact with the DNA template at the DNA site of *Candida albicans* TBP binding. In this assay inhibition is measured as a reduction in the amount of DNA- *Candida albicans* TBP complex produced relative to the amount of DNA- *Candida albicans* TBP complex  
15 produced in the absence of the inhibitor (the positive control). A decrease in the amount of DNA- *Candida albicans* TBP complex is indicative of an inhibitor. Determination of effective levels of DNA- *Candida albicans* TBP inhibition is described below.

One DNA binding assay is constructed as follows. 10 - 100 ng *Candida albicans* TBP, expressed in and purified from *E. Coli* as described above, is incubated  
20 with 0.5 ng labeled (e.g. radioactively or fluorescently labeled) oligonucleotide containing a TATA element such as the one described by Buratowski *et al.* (*Cell* 56, 549-561 (1989)) in reactions containing 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT), 100 µg/ml BSA, 5 - 20 µg/ml poly (dG-dC):(dG-dC) and a candidate inhibitor of complex formation. Reactions are incubated at  
25 30° C for 30-60 min.

Formation of a DNA-TBP complex may be detected as retention of labeled DNA (the label being detected by an appropriate methodology such as scintillation counting for radiolabeled DNA or fluorometry for fluorescently labeled DNA) utilizing known affinity methods for protein immobilization (e.g., biotin/streptavidin, nitrocellulose  
30 filtration, affinity chromatography, immunoaffinity). Nonretention of labeled DNA due to the failure of *Candida albicans* TBP-DNA complex formation is indicative of an effective inhibitor.

Complex formation may also be detected as retention of labeled *Candida albicans* TBP (e.g. radioactively, fluorescently) utilizing known methods for immobilizing DNA. Nonretention of labeled *Candida albicans* TBP due to the failure of *Candida albicans* TBP-DNA complex formation is indicative of an effective inhibitor. These methods are suitable for high-throughput chemical compound library screening applications such as those commonly used in drug discovery.

A third example of detecting DNA/protein complex formation involves detection of an electrophoretic mobility shift of labeled DNA on 4% polyacrylamide gels containing 5% (v/v) glycerol, 25 mM Tris, 100 mM glycine, 1mM EDTA, 5 mM MgCl<sub>2</sub>, pH 8.3 in the presence of *Candida albicans* TBP. The position of the labeled oligonucleotide is detected by appropriate methods (e.g., autoradiography for radioactive oligonucleotide). The absence or deviation of the expected mobility shift due to DNA-*Candida albicans* TBP complex formation is indicative of an effective inhibitor.

Finally, other methods for detecting or separating DNA-protein complexes may be used, including UV crosslinking analysis, high performance liquid chromatography, phage display technology (U.S. Patent No. 5,403,484. Viruses Expressing Chimeric Binding Proteins), fluorescence polarization, and surface plasmon resonance (Biacore, Pharmacia Biosensor, North America) as described below.

### 20 EXAMPLE 3

#### Screening for Inhibition of DNA-Protein Complex Formation

A DNA-protein binding assay consisting of the minimal components necessary to permit DNA-*Candida albicans* TBP association to occur can be used to screen for inhibition of the formation of the DNA-TBP-*Candida albicans* TFIIB complex during transcription<sup>†</sup> initiation. The components of such an assay include: a) a DNA template, b) recombinant *Candida albicans* TBP, c) TFIIB, preferably from *C. albicans*, and optionally d) a candidate *Candida albicans* TBP inhibitor.

The inclusion of an inhibitor molecule that interferes with the interaction between the *Candida albicans* TBP and the DNA template inhibits transcription initiation. The inhibitor may interact directly with the *Candida albicans* TBP protein, and/or it may interact with TFIIB and/or with the DNA template at the site of TFIIB/TBP binding. In this assay inhibition is measured as a reduction in the amount of DNA-TBP-TFIIB complex produced relative to the amount of DNA-TBP-TFIIB complex produced in the

absence of the inhibitor (the positive control). A decrease in the amount of DNA-TBP-TFIIB complex is indicative of an inhibitor. Determination of effective levels of DNA-TBP-TFIIB inhibition is described below.

One DNA binding assay is constructed as follows. 10 - 100 ng *Candida albicans* TBP, expressed in and purified from *E. Coli* as described above, is incubated with 0.5 ng labeled (e.g. radioactively or fluorescently labeled) oligonucleotide containing a TATA element such as the one described by Buratowski *et al.* (*Cell* 56, 549-561 (1989)) and 10 - 100 ng *Candida albicans* TFIIB in reactions containing 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT), 100 µg/ml BSA, 5 - 20 µg/ml poly (dG-dC):(dG-dC) and a candidate inhibitor of complex formation. Reactions are incubated at 30° C for 30-60 min.

Formation of a DNA-TBP-TFIIB complex may be detected as retention of labeled DNA (the label being detected by an appropriate methodology such as scintillation counting for radiolabeled DNA or fluorometry for fluorescently labeled DNA) utilizing known affinity methods for protein immobilization (e.g., biotin/streptavidin, nitrocellulose filtration, affinity chromatography, immunoaffinity). Nonretention of labeled DNA due to the failure of *Candida albicans* TFIIB-TBP-DNA complex formation is indicative of an effective inhibitor.

Complex formation may also be detected as retention of labeled *Candida albicans* TBP (e.g. radioactively, fluorescently) utilizing known methods for immobilizing DNA. Nonretention of labeled *Candida albicans* TBP due to the failure of *Candida albicans* TFIIB-TBP-DNA complex formation is indicative of an effective inhibitor. The preceding two methods are suitable for high-throughput chemical compound library screening applications such as those commonly used in drug discovery.

A third example of detecting DNA/protein complex formation involves detection of an electrophoretic mobility shift of labeled DNA on 4% polyacrylamide gels containing 5% (v/v) glycerol, 25 mM Tris, 100 mM glycine, 1mM EDTA, 5 mM MgCl<sub>2</sub>, pH 8.3 in the presence of *Candida albicans* TFIIB and TBP. The position of the labeled oligonucleotide is detected by appropriate methods (e.g., autoradiography for radioactive oligonucleotide). The absence or deviation of the expected mobility shift due to DNA-*Candida albicans* TBP complex formation is indicative of an effective inhibitor.

Finally, other methods for detecting or separating DNA-protein complexes may be used, including UV crosslinking analysis, high performance liquid

chromatography, phage display technology (U.S. Patent No. 5,403,484. Viruses Expressing Chimeric Binding Proteins), and surface plasmon resonance (Biacore, Pharmacia Biosensor, North America) as described below.

#### 5 EXAMPLE 4

##### Screening for Inhibition of Protein-Protein Complex Formation

A protein-protein binding assay consisting of the minimal components necessary to permit Candida albicans TBP-Candida albicans TFIIB binding to occur can be used to screen for inhibition of the formation of the Candida albicans TBP-Candida  
10 albicans TFIIB complex during transcription initiation. The elements of such an assay consist of; a) recombinant Candida albicans TBP, b) TFIIB, preferably a recombinant Candida albicans TFIIB, and optionally c) a candidate inhibitor of binding.

The inclusion of an inhibitor molecule that interferes with the interaction between the Candida albicans TBP and Candida albicans TFIIB inhibits transcription  
15 initiation. The inhibitor may interact with the Candida albicans TBP or TFIIB protein and thus induce a conformational change which prevents binding, or it may directly inhibit the interaction of Candida albicans TFIIB and TBP proteins. In this assay, inhibition is measured as a reduction in the amount of Candida albicans TBP-TFIIB complex produced relative to the amount of Candida albicans TBP-TFIIB complex produced in the absence  
20 of the inhibitor (the positive control). A decrease in the amount of TFIIB-TBP complex is indicative of an inhibitor. Determination of effective levels of inhibition of Candida albicans TBP-TFIIB binding is described below.

One assay for formation of Candida albicans TBP-TFIIB complex is provided as follows. 10 - 100 ng Candida albicans TFIIB and 10 - 100 ng Candida  
25 albicans TBP are expressed in and purified from *E. coli* as described above, and are added to reactions containing 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 nM dithiothreitol (DTT) 100 µg/ml BSA, and a candidate inhibitor. The mixture is then incubated at 30° C for 30 - 60 min.

Formation of a complex comprising Candida albicans TBP and Candida  
30 albicans TFIIB may be detected by an electrophoretic mobility shift of labeled (e.g. radioactive or fluorescent) TBP or TFIIB on 4% polyacrylamide gels containing 5% (v/v) glycerol, 25 mM Tris, 100 mM glycine, 1mM EDTA, 5 mM MgCl<sub>2</sub>, pH 8.3 in the

presence of the unlabeled partner. The position of the labeled partner is detected by appropriate methods (e.g., autoradiography for radioactive oligonucleotide). The absence or deviation of the expected mobility shift due to Candida albicans TFIIB-TBP complex formation is indicative of an effective inhibitor.

- 5                    Formation of a complex comprising Candida albicans TBP and Candida albicans TFIIB may be detected as retention of labeled TBP utilizing known affinity methods for immobilizing the Candida albicans TFIIB protein (e.g., biotin/streptavidin, nitrocellulose filtration, affinity chromatography, immunoaffinity). The failure of formation of the Candida albicans TFIIB-TBP complex is indicative of inhibition, and is indicated by nonretention of labeled TBP. Alternatively, the immobilized element may be Candida albicans TBP and the labeled partner Candida albicans TFIIB.

- 15                    In the above example, a stronger signal may be conferred in the presence of both TBP and TFIIB and, in addition, a DNA template containing a TATA element. The complex is then quantitated by autoradiography, Phosphorimager technology, or scintillation counting for radioactively labeled factors, fluorometry for fluorescently labeled factors, luminometry for factors labeled with ligands that are detected using chemiluminescent or phosphorescent probing methodologies, or other similar detection methods or materials labeled as described above that are standard in the art.

- 20                    Other methods for detecting or separating protein-protein complexes may be used, including UV crosslinking analysis, high performance liquid chromatography, phage display technology, and surface plasmon resonance as described herein.

#### EXAMPLE 5

##### Assay for Formation of TBP-TFIIB-RNA Polymerase II-DNA Complex

- 25                    Formation of a TBP, TFIIB, RNA polymerase II, DNA complex is known to be markedly stimulated by the addition of another factor, TFIIF. Previous data indicates that TFIIF from *S. cerevisiae* can function in species as distantly related as *Schizosaccharomyces pombe* and humans, strongly suggesting that this factor can functionally replace its *C. albicans* homolog. Accordingly, this factor is purified from *S. cerevisiae* by published methods (Sayre, 1992, J. Biol. Chem. 267:23383) and used to reconstitute formation of a complex containing *C. albicans* TBP, TFIIB, RNA polymerase II and promoter containing DNA such as that described for reconstitution of the TFIIB-TBP-DNA complex.
- 30

Complex formation is carried out in reactions containing, for example, 10 - 100 ng *Candida albicans* TBP, 10 - 100 ng *Candida albicans* TFIIB, 10 - 100 ng *Candida albicans* RNA polymerase II, 10 - 100 ng *S. cerevisiae* TFIIF, 0.5 ng double-stranded TATA element containing-oligonucleotide (same as that used for TFIIB-TBP-DNA complex assay), 10 - 20 mM HEPES (or equivalent), pH 7.5 - 8.0, 5 mM MgCl<sub>2</sub>, 12% glycerol, 10 mM dithiothreitol (DTT), 100 µg/ml BSA, 5 - 20 µg/ml poly (dG-dC); (dG-dC) and compound(s) to be tested for inhibitory activity. Following incubation at 30° C for 30 - 60 min, complexes are detected by one of the methods described above for the TBP-TFIIB-DNA complex. The TBP-TFIIB-RNA polymerase II-DNA complex has a slower electrophoretic mobility than the TBP-TFIIB-DNA complex identified by using the electrophoretic method. In addition, complex formation can be detected as TBP, TFIIB-dependent retention of RNA polymerase II activity (measured by incorporation of labeled nucleotide precursors into acid-insoluble product using the assay for RNA polymerase activity described in the RNA polymerase II purification protocol above) on a matrix with bound TATA-element containing DNA. The IC<sub>50</sub> of inhibitory compounds will be determined by titration into reactions reconstituted as described above. The IC<sub>50</sub> of these compounds against reactions reconstituted with human TBP, TFIIB and RNA polymerase II will also be determined by the same method. Human RNA polymerase II and TFIIF are purified as described previously (Flores et al., 1990, J. Biol. Chem. 265:5629-5634; Reinberg et al., J. Biol. Chem 262:3310-3321). Those compounds whose IC<sub>50</sub> against reactions containing *C. albicans* factors is  $\leq 1/5$  of their IC<sub>50</sub> against reactions reconstituted with human factors will be tested for their ability to inhibit *C. albicans* growth as described below.

## 25 EXAMPLE 6

### Phage Display Inhibitor Screening

In addition to the above mentioned standard techniques of the art, other technologies for molecular identification can be employed in the identification of inhibitor molecules. One of these technologies is phage display technology (U.S. Patent No. 5,403,484. Viruses Expressing Chimeric Binding Proteins). Phage display permits identification of a binding protein against a chosen target. Phage display is a protocol of molecular screening which utilizes recombinant bacteriophage. The technology involves transforming bacteriophage with a gene that encodes an appropriate ligand (in this case,

a candidate inhibitor) capable of binding to the target molecule of interest. For the purposes of this disclosure, the target molecule may be *Candida albicans* TBP, or a DNA-protein or protein-protein complex formed using TBP and/or TFIIB, as described herein. The transformed bacteriophage (which preferably is tethered to a solid support) express  
5 the candidate inhibitor and display it on their phage coat. The cells or viruses bearing the candidate inhibitor which recognize the target molecule are isolated and amplified. The successful inhibitors are then characterized.

Phage display technology has advantages over standard affinity ligand screening technologies. The phage surface displays the microprotein ligand in a three  
10 dimensional conformation, more closely resembling its naturally occurring conformation. This allows for more specific and higher affinity binding for screening purposes.

### EXAMPLE 7

#### Biospecific Interaction Analysis

15 A second relatively new screening technology which may be applied to the inhibitor screening assays of this invention is biospecific interaction analysis (BIAcore, Pharmacia Biosensor AB, Uppsala, Sweden). This technology is described in detail by Jonsson *et al.* (Biotechniques 11:5, 620-627 (1991)). Biospecific interaction analysis utilizes surface plasmon resonance (SPR) to monitor the adsorption of biomolecular  
20 complexes on a sensor chip. SPR measures the changes in refractive index of a polarized light directed at the surface of the sensor chip.

Specific ligands (i.e., candidate inhibitors) capable of binding to the target molecule of interest (i.e., *Candida albicans* TBP or a protein-protein or protein-DNA complex containing TBP) are immobilized to the sensor chip. In the presence of the  
25 target molecule, specific binding to the immobilized ligand occurs. The nascent immobilized ligand-target molecule complex causes a change in the refractive index of the polarized light and is detected on a diode array. Biospecific interaction analysis provides the advantages of; 1) allowing for label-free studies of molecular complex formation; 2) studying molecular interactions in real time as the assay is passed over the sensor chip;  
30 3) detecting surface concentrations down to 10 pg/mm<sup>2</sup>; detecting interactions between two or more molecules; and 4) being fully automated (Biotechniques 11:5, 620-627 (1991)).

## EXAMPLE 8

### High Throughput Screening of Potential Inhibitors

It is contemplated according to the invention that the screening methods disclosed herein encompass screening of multiple samples simultaneously, also referred to herein as 'high throughput' screening. For example, in high throughput screening, from several hundred to several thousand candidate inhibitors may be screened in a single assay. Several examples of high throughput screening assays useful according to the invention are as follows.

A protein A (pA)-*C. albicans* TBP fusion protein is generated by inserting the coding sequence of TBP in frame downstream of the pA coding sequence of the plasmid pRIT2T (Pharmacia Biotech). The fusion construct is induced, and the resultant recombinant protein is extracted and purified according to the manufacturer's recommended conditions. This procedure can also be carried out for the preparation of a pA-*Candida albicans* TFIIB fusion protein except that the downstream coding sequence is that of TFIIB protein; all other steps would remain the same.

A Dynatech Microlite 2 microtiter plate or equivalent high protein-binding capacity plate is coated with 1  $\mu\text{g}/\text{well}$  human IgG by incubating 300  $\mu\text{l}$  3.33  $\mu\text{g}/\text{ml}$  human IgG (Sigma) in coating buffer (0.2 M sodium carbonate, pH 9.4) in the well for 4-12 hr at 4°C. The coating buffer is then decanted and the wells are washed five times with 300  $\mu\text{l}$  PBS. 300  $\mu\text{l}$  blocking buffer (SuperBlock™ blocking buffer; Pierce) containing 3.33  $\mu\text{g}/\text{ml}$  pA-TBP or pA-TFIIB are added and the plate is incubated for 4 or more hours at 4°C. The plates may be stored in this form at 4°C until ready for use. When ready for use the plates are washed five times with 300  $\mu\text{l}$  PBS. Test compound at a final concentration of 20-200  $\mu\text{M}$ , labeled TBP or TFIIB (i.e., nonfusion protein), whichever is not added during the coating step, and 10 - 1000 fmol promoter-containing oligonucleotides are suspended in HEG buffer containing 200  $\mu\text{g}/\text{ml}$  BSA in a total volume of 150  $\mu\text{l}$  and are added and the reaction is incubated at room temperature with gentle agitation for 60 min. The plate is then washed five times with PBS using a Dynatech plate washer or equivalent. Bound labeled protein is quantitated by adding 250  $\mu\text{l}$  Microscint (Packard) per well and is counted in a microtiter plate-compatible scintillation spectrophotometer.

As an alternative, the protein A fusion and the second, non-fusion protein can be incubated in the presence of test compound in polypropylene microtiter plates

under the same buffer and incubation conditions described above. The reaction mix is then transferred to the wells of a microtiter plate coated with human IgG (which is prepared as described above, and is stored in blocking buffer and is washed five times with 300  $\mu$ l PBS immediately before use) and is incubated for 60 min at room temperature with gentle  
5 agitation. Retention of radioactive protein is quantified as above.

Interaction of TBP and TFIIB, which is measured as retention of radioactivity on the plate, is dependent on human IgG coating the plate and wild-type Candida albicans TBP or TFIIB, one of which must be fused to pA. Candidate inhibitors or extracts that inhibit retention of radioactivity by more than 30% are identified  
10 and the inhibitory activity is further purified if necessary.

Inhibitors identified as described above are then tested for their ability to inhibit Candida albicans TBP-dependent transcription in an *in vitro* transcription system as described herein, and also may be tested for their ability to inhibit Candida albicans growth.

15 Other fusion or modified protein systems that are contemplated include, but are not limited to, glutathione-S-transferase, maltose binding protein, influenza virus hemagglutinin, FLAG™ and hexahistidine fusions to Candida albicans TBP or Candida albicans TFIIB which are prepared, expressed, and purified by published methods or biotinylated Candida albicans TBP or TFIIB which are prepared using reactive biotin  
20 precursors available commercially. The purified fusion or modified protein is immobilized on a microtiter plate containing the appropriate ligand for each fusion protein (e.g. glutathione, amylose, CA157 antibody, etc., respectively) and the assay is carried out and the results evaluated in essentially the same manner as described above.

## 25 EXAMPLE 9

### Candidate Inhibitors

A "candidate inhibitor," as used herein, is any compound with a potential to inhibit Candida albicans TBP-mediated transcription initiation or complex formation.

A candidate inhibitor is tested in a concentration range that depends upon the molecular  
30 weight of the molecule and the type of assay. For example, for inhibition of protein/protein or protein/DNA complex formation or transcription initiation, small molecules (as defined below) may be tested in a concentration range of 1pg - 100 ug/ml, preferably at about 100 pg - 10 ng/ml; large molecules, e.g., peptides, may be tested in

the range of 10 ng - 100 ug/ml, preferably 100 ng - 10 ug/ml.

Inhibitors of *Candida albicans* growth or viability may target the novel transcription factor described herein, TBP, or it may target a protein or nucleic acid that interacts with the novel transcription factor so as to prevent the natural biological interaction that occurs *in vivo* and leads to transcription initiation in *Candida*. Thus, an inhibitor identified as described herein will possess two properties: 1) at some concentration it will inhibit *Candida albicans* growth or viability; and 2) at the same concentration, it will not significantly affect the growth of mammalian, particularly human, cells.

Candidate inhibitors will include peptide and polypeptide inhibitors having an amino acid sequence based upon the novel TBP sequences described herein. For example, a fragment of TBP may act as a competitive inhibitor with respect to TBP binding to other proteins involved in *Candida* transcription, e.g., RNA polymerase II, TFIIB, or with respect to binding of the transcription complex to the DNA template.

Candidate inhibitor compounds from large libraries of synthetic or natural compounds can be screened. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, WI). Combinatorial libraries are available and can be prepared. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g., Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily produceable. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means.

Useful compounds may be found within numerous chemical classes, though typically they are organic compounds, and preferably small organic compounds. Small organic compounds have a molecular weight of more than 50 yet less than about 2,500 daltons, preferably less than about 750, more preferably less than about 350 daltons. Exemplary classes include heterocycles, peptides, saccharides, steroids, and the like. The compounds may be modified to enhance efficacy, stability, pharmaceutical compatibility, and the like. Structural identification of an agent may be used to identify, generate, or

screen additional agents. For example, where peptide agents are identified, they may be modified in a variety of ways to enhance their stability, such as using an unnatural amino acid, such as a D-amino acid, particularly D-alanine, by functionalizing the amino or carboxylic terminus, e.g. for the amino group, acylation or alkylation, and for the  
 5 carboxyl group, esterification or amidification, or the like. Other methods of stabilization may include encapsulation, for example, in liposomes, etc.

### EXAMPLE 10

#### Measurement for effective inhibition

10 The amount of inhibition by a candidate inhibitor is quantified using the following formula, which describes reactions reconstituted with a radioactively labeled moiety.

$$15 \quad \text{Percent Inhibition} = \frac{(\text{CPM}_{\text{Positive Control}} - \text{CPM}_{\text{Sample}})}{(\text{CPM}_{\text{Positive Control}})} \times 100$$

where  $\text{CPM}_{\text{Positive Control}}$  is the average of the cpm in complexes or RNA molecules formed in reactions that lack the candidate inhibitor, and  $\text{CPM}_{\text{Sample}}$  is the cpm in complexes  
 20 formed in reactions containing the candidate inhibitor. Candidate inhibitors for which the percent inhibition is 50% are titrated into reactions containing either Candida albicans TBP or human TBP (expressed in and purified from *E. coli* using existing recombinant clones (Peterson et al., *Science* 248, 1625-1630, 1990; Kao et al., *Science* 248, 1646-1650, 1990; Hoffman, et al., *Nature* 346, 387-390, 1990, and assayed as described above)  
 25 and their  $\text{IC}_{50}$  with respect to human and Candida albicans TBP determined from graphs of compound concentration vs. % inhibition. The  $\text{IC}_{50}$  is defined as the concentration that results in 50% inhibition. Candidate inhibitors for which the  $\text{IC}_{50}$  against Candida albicans TBP-containing reactions is less than or equal to 1/5 the  $\text{IC}_{50}$  against human TBP-containing reactions are further tested for their ability to inhibit the growth of Candida  
 30 albicans in culture as described below.

EXAMPLE 11Measurement for inhibition of *Candida albicans* growth in culture

Once an inhibitor is identified in one or more of the binding or transcription assays described herein, it may be desirable to determine the effect of the inhibitor on the growth and/or viability of *Candida albicans* in culture. A candidate inhibitor is tested for its ability to inhibit growth of *Candida albicans* cells in culture as follows. Methods for performing tests on growth inhibition in culture are well-known in the art. Once such procedure is based on the NCCLS M27P method (The National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; proposed standard, 1992), as follows. Serial dilutions (two- or three-fold steps starting from a maximum concentration of 100 - 200  $\mu\text{g/ml}$ ) of candidate inhibitor are prepared using RPMI-1640 medium as diluent and an aliquot of 100  $\mu\text{l}$  of each dilution is added to the wells of a 96-well polystyrene microtiter plate. Five *Candida albicans* colonies, picked from a Sabouraud Dextrose Agar plate inoculated 14-20 hr previously with the test *Candida albicans* strain (Catalog number 10231 from the American Type Culture Collection Yeast Catalog), are resuspended in RPMI-1640 medium such that the density of cells is 10,000 - 30,000 cells/ml. 100  $\mu\text{l}$  of the cell suspension is added to each of the wells of the 96-well microtiter plate containing diluted candidate inhibitor and medium control. Cultures are mixed by agitation and incubated at 35° C for 48 hr. without agitation, after which cell growth is monitored by visual inspection for the formation of turbidity and/or mycelial colonies. The minimum concentration of candidate inhibitor at which no cell growth is detected by this method is defined as the minimum inhibitory concentration (MIC) for that compound. Examples of MICs for known antifungal compounds obtained using this technique are 0.125 - 0.5  $\mu\text{g/ml}$  for fluconazole and 0.25 - 1.0  $\mu\text{g/ml}$  for amphotericin B (The National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; proposed standard, 1992). An inhibitor identified by the methods described herein, will have MIC which is equivalent to or less than the MICs for fluconazole or amphotericin B.

## EXAMPLE 12

### Transcription Inhibition Counterscreen Using Human TBP

A compound identified as an inhibitor of *Candida albicans* according to one or more of the assays described herein may be tested further in order to determine its effect on the host organism. In the development of useful antifungal compounds for human therapeutics, it is desirable that such compounds act as effective agents in inhibiting the viability of the fungal pathogen while not significantly inhibiting human cell systems. Specifically, inhibitors of *Candida albicans* identified in any one of the above described assays may be counterscreened for inhibition of human TBP.

Recombinant human TBP can be obtained from existing sources and purified by published methods (for example, see Peterson et al., Kao et al., and Hoffman et al., supra) and contacted with the candidate inhibitor in assays such as those described above but using a human system. The effectiveness of a *Candida albicans* TBP inhibitor as a human therapeutic is determined as one which exhibits a low level of inhibition against human TBP relative to the level of inhibition with respect to *Candida albicans* TBP. For example, it is preferred that the amount of inhibition by a given inhibitor of human TBP in a human system be no more than 20% with respect to the amount of inhibition of *Candida albicans* TBP/TFIB in a *Candida* system when tested in any of the assays described above.

### Dosage and Pharmaceutical Formulations

For therapeutic uses, inhibitors identified as described herein may be administered in a pharmaceutically acceptable/biologically compatible formulation, for example, in the form of a cream, ointment, lotion or spray for topical use, or in a physiological solution, such as a salt solution, for internal administration. The amount of inhibitor administered will be determined according to the degree of pathogenic infection and whether the infection is systemic or localized, and will typically be in the range of about 1 µg - 100 mg/kg body weight. Where the inhibitor is a peptide or polypeptide, it will be administered in the range of about 100 - 500 µg/ml per dose. A single dose of inhibitor or multiple doses, daily, weekly, or intermittently, is contemplated according to the invention.

The route of administration will be chosen by the physician, and may be topical, oral, transdermal, nasal, rectal, intravenous, intramuscular, or subcutaneous.

Budapest Treaty Deposit

*E. coli* transformed with a plasmid containing the gene encoding *Candida albicans* TBP has been deposited in an international depository, the A.T.C.C., Rockville, MD, under the accession number 69900, on September 15, 1995. *E. coli* transformed  
5 with a plasmid containing the gene encoding *Candida albicans* TFIIB has been deposited in an international depository, the A.T.C.C., Rockville, MD, under the accession number 69899, on September 15, 1995. A.T.C.C. Nos. 69900 and 69899 will be available to the public upon the grant of a patent which discloses the accession numbers in conjunction with the invention described herein. The deposits were made under the Budapest Treaty,  
10 will be available beyond the enforceable life of the patent for which the deposit is made, and will be maintained for a period of at least 30 years from the time of deposit and at least 5 years after the most recent request for the furnishing of a sample of the deposit is received by the A.T.C.C. It is to be understood that the availability of these deposits does not constitute a license to practice the subject invention in derogation of patent rights  
15 granted for the subject invention by governmental action.

OTHER EMBODIMENTS

The foregoing examples demonstrate experiments performed and contemplated by the present inventors in making and carrying out the invention. It is believed that these examples include a disclosure of techniques which serve to both apprise  
20 the art of the practice of the invention and to demonstrate its usefulness. It will be appreciated by those of skill in the art that the techniques and embodiments disclosed herein are preferred embodiments only that in general numerous equivalent methods and techniques may be employed to achieve the same result.

All of the references identified hereinabove, are hereby expressly  
25 incorporated herein by reference to the extent that they describe, set forth, provide a basis for or enable compositions and/or methods which may be important to the practice of one or more embodiments of the present inventions.

5

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

10

(i) APPLICANT: SCRIPTGEN PHARMACEUTICALS, INC.

(ii) TITLE OF THE INVENTION: NOVEL TATA-BINDING PROTEIN FROM CANDIDA ALBICANS, NUCLEIC ACID SEQUENCE CODING THEREFORE, AND METHODS OF SCREENING FOR INHIBITORS OF CANDIDA ALBICANS GROWTH

15

(iii) NUMBER OF SEQUENCES: 4

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(F) ZIP: 10022-7513

25

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

30

(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

35

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/626,309

(B) FILING DATE: 01-APR-1996

40

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55

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 219 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

60

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

65

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 Ile Ile Lys Lys Glu Asp Ile Asp Glu Pro Lys Gln Glu Asp Thr Thr  
 20 25 30  
 Asp Ser Asn Gly Gly Gly Gly Ile Gly Ile Val Pro Thr Leu Gln Asn  
 35 40 45  
 Ile Val Ala Thr Val Asn Leu Asp Cys Arg Leu Asp Leu Lys Thr Ile  
 50 55 60  
 10 Ala Leu His Ala Arg Asn Ala Glu Tyr Asn Pro Lys Arg Phe Ala Ala  
 65 70 75 80  
 Val Ile Met Arg Ile Arg Asp Pro Lys Thr Thr Ala Leu Ile Phe Ala  
 85 90 95  
 15 Ser Gly Lys Met Val Val Thr Gly Ala Lys Ser Glu Asp Asp Ser Lys  
 100 105 110  
 Leu Ala Ser Arg Lys Tyr Ala Arg Ile Ile Gln Lys Leu Gly Phe Asn  
 115 120 125  
 Ala Lys Phe Cys Asp Phe Lys Ile Gln Asn Ile Val Gly Ser Thr Asp  
 130 135 140  
 20 Val Lys Phe Ala Ile Arg Leu Glu Gly Leu Ala Phe Ala His Gly Thr  
 145 150 155 160  
 Phe Ser Ser Tyr Glu Pro Glu Leu Pro Pro Gly Leu Ile Tyr Arg Met  
 165 170 175  
 25 Val Lys Pro Lys Ile Val Leu Leu Ile Phe Val Ser Gly Lys Ile Val  
 180 185 190  
 Leu Thr Gly Ala Lys Lys Arg Glu Glu Ile Tyr Asp Ala Phe Glu Ser  
 195 200 205  
 Ile Tyr Pro Val Leu Asn Glu Phe Arg Lys Asn  
 210 215

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 344 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

50 Met Ser Pro Ser Thr Ser Thr Ala Val Gln Glu Tyr Ile Gly Pro Asn  
 1 5 10 15  
 Leu Asn Val Thr Leu Thr Cys Pro Glu Cys Lys Ile Phe Pro Pro Asp  
 20 25 30  
 Leu Val Glu Arg Phe Ser Glu Gly Asp Ile Val Cys Gly Ser Cys Gly  
 35 40 45  
 55 Leu Val Leu Ser Asp Arg Val Val Asp Thr Arg Ser Glu Trp Arg Thr  
 50 55 60  
 Phe Ser Asn Asp Asp Gln Asn Gly Asp Asp Pro Ser Arg Val Gly Asp  
 65 70 75 80  
 Ala Gly Asn Pro Leu Leu Asp Thr Glu Asp Leu Ser Thr Met Ile Ser  
 85 90 95  
 60 Tyr Ala Pro Asp Ser Thr Lys Ala Gly Arg Glu Leu Ser Arg Ala Gln  
 100 105 110  
 Ser Lys Ser Leu Val Asp Lys Lys Asp Asn Ala Leu Ala Ala Tyr  
 115 120 125  
 65 Ile Lys Ile Ser Gln Met Cys Asp Gly Tyr Gln Leu Pro Lys Ile Val  
 130 135 140  
 Ser Asp Gly Ala Lys Glu Val Tyr Lys Met Val Tyr Asp Glu Lys Pro  
 145 150 155 160

Leu Arg Gly Lys Ser Gln Glu Ser Ile Met Ala Ala Ser Ile Phe Ile  
 165 170 175  
 Gly Cys Arg Lys Ala Asn Val Ala Arg Ser Phe Lys Glu Ile Trp Ala  
 180 185 190  
 5 Lys Thr Asn Val Pro Arg Lys Glu Ile Gly Lys Val Phe Lys Ile Met  
 195 200 205  
 Asp Lys Ile Ile Arg Glu Lys Asn Ala Ala Asn Pro Asn Ala Ala Tyr  
 210 215 220  
 10 Tyr Gly Gln Asp Ser Ile Gln Thr Thr Gln Thr Ser Ala Glu Asp Leu  
 225 230 235 240  
 Ile Arg Arg Phe Cys Ser His Leu Gly Val Asn Thr Gln Val Thr Asn  
 245 250 255  
 Gly Ala Glu Tyr Ile Ala Arg Arg Cys Lys Glu Val Gly Val Leu Ala  
 260 265 270  
 15 Gly Arg Ser Pro Thr Thr Ile Ala Ala Thr Val Ile Tyr Met Ala Ser  
 275 280 285  
 Leu Val Phe Gly Phe Asp Leu Pro Pro Ser Lys Ile Ser Asp Lys Thr  
 290 295 300  
 20 Gly Val Ser Asp Gly Thr Ile Lys Thr Ser Tyr Lys Tyr Met Tyr Glu  
 305 310 315 320  
 Glu Lys Glu Gln Leu Ile Asp Pro Ser Trp Ile Glu Ser Gly Lys Val  
 325 330 335  
 Lys Leu Glu Lys Ile Pro Lys Asn  
 340

25

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 657 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 35 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

40 (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAAGTCAA TAGAGGAAGA TGAAAAAAT AAAGCCGAAG ATTTGGATAT TATAAAAAAC 60  
 GAAGATATTG ATGAACCTAA ACAAGAAGAT ACCACTGATA GTAATGGTGG TGGAGGTATT 120  
 45 GGTATAGTGC CCACATTACA AAATATTCTT GCTACGGTGA ATCTTGATTG TCGACTTGAT 180  
 AAAACAATTG CTTTACATGC TAGAAATGCC GAATATAATC CAAAACGTTT TGCTGCGGTG 240  
 ATTATGAGAA TTAGAGATCC AAAAATACTG GCATTAATCT TTGCTTCGGG GAAAATGGTT 300  
 GTGACTGGGG CTAAATCCGA AGACGATTCC AAGTTGGCTT CAAGAAAGTA TGCTAGAATC 360  
 ATTCAAAAGT TGGGGTTCAA TGCTAAATTT TGTGATTTTA AAATTCAAAA TATAGTGGGG 420  
 50 TCAACAGATG TTAAGTTTGC TATTAGATTA GAAGGCTTAG CTTTGTCTCA TGGTACTTTT 480  
 TCTTCATATG AACCAGAATT ATTTCTGGG TTAATTTATA GAATGGTGAA ACCAAAAATT 540  
 GTTTTACTTA TATTTGTTTC TGGGAAAATT GTTTTGACGG GTGCCAAAAA GACAGAAGAA 600  
 ATTTATGATG CATTTGAACT GATTTATCCG GTTTTAAATG AATTCGTAA AAATTGA 657

55

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 1095 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY:

(ii) MOLECULE TYPE:  
(iii) HYPOTHETICAL: NO  
(iv) ANTISENSE: NO  
(v) FRAGMENT TYPE:  
(vi) ORIGINAL SOURCE:

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

10	TAAGCTTGTA	TTACTAAGCA	TATTATGTCG	CCATCAACAT	CTACGGCAGT	ACAGGAGTAT	60
	ATTGGACCAA	ACTTGAATGT	TACATTAACA	TGTCCTGAGT	GTAAGATATT	TCCACCTGAT	120
	TTGGTAGAGA	GGTTCAGCGA	AGGTGACATT	GTCTGTGGCA	GTTGTGGGCT	AGTATTGAGT	180
	GATCGTGTTG	TGGATACGAG	ATCAGAATGG	AGAACTTTCA	GTAACGATGA	CCAAAATGGT	240
	GATGATCCTT	CTCGTGTTGG	TGATGCAGGT	AACCCTTTAT	TAGACACAGA	GGACTTGTC	300
15	ACAATGATTT	CTTATGCTCC	TGATACTACC	AAAGCAGGAA	GAGAGTTAAG	CCGAGCCCCA	360
	TCTAAATCTC	TAGTCGATAA	AAAAGACAAT	GCATTGGCTG	CAGCATATAT	CAAGATTTCT	420
	CAAATGTGCG	ATGGTTATCA	ATTGCCTAAA	ATAGTTCTGG	ATGGGGCCAA	GGAAGTCTAC	480
	AAAATGGTTT	ATGACGAGAA	ACCATTGCGA	GGAAAATCAC	AAGAGAGTAT	CATGGCAGCT	540
	TCTATCTTTA	TTGGTTGCAG	AAAGGCCAAT	GTTGCTCGTT	CATTCAAAGA	GATATGGGCA	600
	AAGACTAATG	TACCTCGTAA	GGAAATTGGT	AAAGTGTTCA	AGATCATGGA	CAAGATCATT	660
20	CGTGAAAAGA	ATGCAGCCAA	CCCTAATGCT	GCATATTACG	GTCAAGACAG	CATTCAAACC	720
	ACCCAAACTT	CGGCCGAGGA	TTTGATTAGA	AGATTCTGTT	CTCACTTGGG	TGTTAACACA	780
	CAAGTTACAA	ATGGTGCGGA	ATACATAGCC	AGAAGATGTA	AGGAAGTCGG	GGTTTTAGCA	840
	GGTAGATCGC	CAACTACAAT	TGCTGCAACT	GTAATTTACA	TGGCTTCACT	AGTGTTTGGA	900
	TTTGACTTAC	CTCCATCCAA	GATATCTGAT	AAAACCTGGT	TCAGTGATGG	TACTATCAAA	960
25	ACTTCATACA	AGTACATGTA	CGAGGAGAAA	GAACAATTGA	TTGATCCATC	TTGGATAGAA	1020
	AGTGGTAAAG	TAAAATTGGA	AAAAATACCA	AAAAACTAAT	ACAGCGGAGT	CGCCACTGTT	1080
	AATCCTTTAC	CCTCT					1095

CLAIMS

- 1                   1.     A recombinant nucleic acid comprising a nucleic acid sequence  
2     encoding Candida albicans TBP.
- 1                   2.     A vector comprising a nucleic acid sequence encoding Candida  
2     albicans TBP.
- 1                   3.     A transformed host cell containing a nucleic acid sequence encoding  
2     Candida albicans TBP.
- 1                   4.     A recombinant polypeptide comprising Candida albicans TBP.
- 1                   5.     A fragment of Candida albicans TBP, said fragment being  
2     characterized in that it inhibits the biological activity of Candida albicans TBP in  
3     transcription initiation.
- 1                   6.     A fragment of Candida albicans TBP, said fragment being  
2     characterized in that it prevents the growth of Candida albicans.
- 1                   7.     A method for producing recombinant Candida albicans TBP,  
2     comprising culturing the host cell of claim 3 under conditions sufficient to permit  
3     expression of the nucleic acid encoding Candida albicans TBP, and isolating said Candida  
4     albicans TBP.
- 1                   8.     A screening method for identifying an inhibitor of Candida albicans  
2     growth, comprising detecting inhibition of mRNA transcription in an in vitro transcription  
3     assay comprising a DNA template, RNA polymerase II, recombinant Candida albicans  
4     TBP, and a candidate inhibitor, wherein production of an mRNA transcript from said  
5     DNA template occurs in the absence of said candidate inhibitor.

1                   9.     A screening method for identifying an inhibitor of Candida albicans  
2 growth, comprising detecting in the presence of a candidate inhibitor inhibition of  
3 formation of a complex comprising a DNA template and recombinant Candida albicans  
4 TBP, wherein in the absence of said candidate inhibitor, formation of said complex  
5 occurs.

1                   10.    A screening method for identifying an inhibitor of Candida albicans  
2 growth, comprising detecting in the presence of a candidate inhibitor inhibition of  
3 formation of a complex comprising Candida albicans TFIIB and Candida albicans TBP,  
4 wherein in the absence of said candidate inhibitor formation of said complex occurs.

1                   11.    A screening method for identifying an inhibitor of Candida albicans  
2 growth, comprising detecting in the presence of a candidate inhibitor inhibition of  
3 formation of a complex comprising RNA polymerase II, Candida albicans TBP, and  
4 Candida albicans TFIIB, wherein in the absence of said candidate inhibitor formation of  
5 said complex occurs.

1                   12.    The screening method of claim 8, 9, 10 or 11, wherein said  
2 detecting is performed in the presence of a plurality of candidate inhibitors such that said  
3 inhibition is indicative of inhibition by a said candidate inhibitor of said plurality.

1                   13.    The screening method of claim 8, 9, 10, or 11, wherein multiple  
2 detecting steps are performed simultaneously using a plurality of candidate inhibitors,  
3 wherein detection of inhibition by any one candidate inhibitor is detectable independently  
4 of said plurality.

1                   14.    A method of preventing Candida albicans growth in culture,  
2 comprising contacting said culture with an inhibitor that selectively inhibits the biological  
3 activity of Candida albicans TBP.

1                   15.    A method of preventing Candida albicans growth in a mammal,  
2 comprising administering to said mammal a therapeutically effective amount of an  
3 inhibitor that inhibits the biological activity of Candida albicans TBP.

## FIG. 1A

1	ATG	GAT	TTA	AAA	TTA	CCC	CCA	ACT	AAT	CCA	ACC	AAC	CCA	CAA	GCA	AAG	ACT	TTT	ATG	60	
1	M	D	L	K	L	P	P	T	N	P	T	N	P	Q	A	K	T	F	M	20	
61	AAG	TCA	ATA	GAG	GAA	GAT	GAA	AAA	AAT	AAA	GCC	GAA	GAT	TTG	GAT	ATT	ATA	AAA	AAG	GAA	120
21	K	S	I	E	E	D	E	K	N	K	A	E	D	L	D	I	I	K	K	E	40
121	GAC	ATT	GAT	GAA	CCT	AAA	CAA	GAA	GAT	ACC	ACT	GAT	AGT	AAT	GGT	GGT	GGT	GGT	ATT	GGT	180
41	D	I	D	E	P	K	Q	E	D	T	T	D	S	N	G	G	G	I	G	60	
181	ATA	GTG	CCC	ACA	TTA	CAA	AAT	ATT	GTT	GCT	ACG	GTG	AAT	CTT	GAT	TGT	CGA	CTT	GAT	CTT	240
61	I	V	P	T	L	Q	N	I	V	A	T	V	N	L	D	C	R	L	D	L	80
241	AAA	ACA	ATT	GCT	TTA	CAT	GCT	AGA	AAT	GCC	GAA	TAT	AAT	CCA	AAA	CGT	TTT	GCT	GCG	GTG	300
81	K	T	I	A	L	H	A	R	N	A	E	Y	N	P	K	R	F	A	A	V	100
301	ATT	ATG	AGA	ATT	AGA	GAT	CCA	AAA	ACT	ACG	GCA	TTA	ATC	TTT	GCT	TCG	GGG	AAA	ATG	GTT	360
101	I	M	R	I	R	D	P	K	T	T	A	L	I	F	A	S	G	K	M	V	120

A

A

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## FIG. 1B

A ————— A

361	GTG	ACT	GGG	GCT	AAA	TCC	GAA	GAT	GAT	TCC	AAG	TTG	GCT	TCA	AGA	AAG	TAT	GCT	AGA	ATC	420
121	V	T	G	A	K	S	E	D	D	S	K	L	A	S	R	K	Y	A	R	I	140
421	ATT	CAA	AAG	TTG	GGG	TTC	AAT	GCT	AAA	TTT	TGT	GAT	TTT	AAA	ATT	CAA	AAT	ATA	GTG	GGG	480
141	I	Q	K	L	G	F	N	A	K	F	C	D	F	K	I	Q	N	I	V	G	160
481	TCA	ACA	GAT	GTT	AAG	TTT	GCT	ATT	AGA	TTA	GAA	GGC	TTA	GCT	TTT	GCT	CAT	GGT	ACT	TTC	540
161	S	T	D	V	K	F	A	I	R	L	E	G	L	A	F	A	H	G	T	F	180
541	TCT	TCA	TAT	GAA	CCA	GAA	TTA	TTT	CCT	GGG	TTA	ATT	TAT	AGA	ATG	GTG	AAA	CCA	AAA	ATT	600
181	S	S	Y	E	P	E	L	F	P	G	L	I	Y	R	M	V	K	P	K	I	200
601	GTT	TTA	CTT	ATA	TTT	GTT	TCT	GGG	AAA	ATT	GTT	TTG	ACG	GGT	GCC	AAA	AAG	AGA	GAA	GAA	660
201	V	L	L	I	F	V	S	G	K	I	V	L	T	G	A	K	K	R	E	E	220
661	ATT	TAT	GAT	GCA	TTT	GAA	CTG	ATT	TAT	CCG	GTT	TTA	AAT	GAA	TTT	CGT	AAA	AAT	TGA		717
221	I	Y	D	A	F	E	<b>S</b>	I	Y	P	V	L	N	E	F	R	K	N	*		239

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## FIG. 2A

1 TAAGCTTGTAATAAGCATATT ATG TCG CCA TCA ACA TCT ACG GCA GTA CAG GAG TAT ATT GGA 66  
 1 M S P S T S T A V Q E Y I G 14  
 67 CCA AAC TTG AAT GTT ACA TTA ACA TGT CCT GAG TGT AAG ATA TTT CCA CCT GAT TTG GTA 126  
 15 P N L N V T L T C P E C K I F P P D L V 34  
 127 GAG AGG TTC AGC GAA GGT GAC ATT GTC TGT GGC AGT TGT GGG CTA GTA TTG AGT GAT CGT 186  
 35 E R F S E G D I V C G S C G L V L S D R 54  
 187 GTT GTG GAT ACG AGA TCA GAA TGG AGA ACT TTC AGT AAC GAT GAC CAA AAT GGT GAT GAT 246  
 55 V V D T R S E W R T F S N D D Q N G D D 74  
 247 CCT TCT CGT GTT GGT GAT GCA GGT AAC CCT TTA TTA GAC ACA GAG GAC TTG TCC ACA ATG 306  
 75 P S R V G D A G N P L L D T E D L S T M 94  
 307 ATT TCT TAT GCT CCT GAT AGT ACC AAA GCA GGA AGA GAG TTA AGC CGA GCC CAA TCT AAA 366  
 95 I S Y A P D S T K A G R E L S R A Q S K 114  
 367 TCT CTA GTC GAT AAA AAA GAC AAT GCA TTG GCT GCA GCA TAT ATC AAG ATT TCT CAA ATG 426  
 115 S L V D K K D N A L A A A Y I K I S Q M 134  
 427 TGC GAT GGT TAT CAA TTG CCT AAA ATA GTT CTG GAT GGG GCC AAG GAA GTC TAC AAA ATG 486  
 135 C D G Y Q L P K I V **S** D G A K E V Y K M 154  
 487 GTT TAT GAC GAG AAA CCA TTG CGA GGA AAA TCA CAA GAG AGT ATC ATG GCA GCT TCT ATC 546  
 155 V Y D E K P L R G K S Q E S I M A A S I 174

3/4

A\_\_\_\_\_A

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## FIG. 2B

A ————— A

547 TTT ATT GGT TGC AGA AAG GCC AAT GTT GCT CGT TCA TTC AAA GAG ATA TGG GCA AAG ACT 606  
 175 F I G C R K A N V A R S F K E I W A K T 194  
  
 607 AAT GTA CCT CGT AAG GAA ATT GGT AAA GTG TTC AAG ATC ATG GAC AAG ATC ATT CGT GAA 666  
 195 N V P R K E I G K V F K I M D K I I R E 214  
  
 667 AAG AAT GCA GCC AAC CCT AAT GCT GCA TAT TAC GGT CAA GAC AGC ATT CAA ACC ACC CAA 726  
 215 K N A A N P N A A Y Y G Q D S I Q T T Q 234  
  
 727 ACT TCG GCC GAG GAT TTG ATT AGA AGA TTC TGT TCT CAC TTG GGT GTT AAC ACA CAA GTT 786  
 235 T S A E D L I R R F C S H L G V N T Q V 254 <sup>4/4</sup>  
  
 787 ACA AAT GGT GCG GAA TAC ATA GCC AGA AGA TGT AAG GAA GTC GGG GTT TTA GCA GGT AGA 846  
 255 T N G A E Y I A R R C K E V G V L A G R 274  
  
 847 TCG CCA ACT ACA ATT GCT GCA ACT GTA ATT TAC ATG GCT TCA CTA GTG TTT GGA TTT GAC 906  
 275 S P T I A A T V I Y M A S L V F G F D 294  
  
 907 TTA CCT CCA TCC AAG ATA TCT GAT AAA ACT GGT GTC AGT GAT GGT ACT ATC AAA ACT TCA 966  
 295 L P P S K I S D K T G V S D G T I K T S 314  
  
 967 TAC AAG TAC ATG TAC GAG GAG AAA CAA TTG ATT GAT CCA TCT TGG ATA GAA AGT GGT 1026  
 315 Y K Y M Y E E K E Q L I D P S W I E S G 334  
  
 1027 AAA GTA AAA TTG GAA AAA ATA CCA AAA AAC TAA TACAGCGAGTCCACCTGTTAATCCTTTACCCCTCT 1095  
 335 K V K L E K I P K N \* 345

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/06170

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) Please See Extra Sheet.

US CL Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. 435/6, 7, 8, 29, 69, 1, 70, 1, 71, 1, 243, 320, 1, 325; 530/350, 536/23, 74

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PETERSON et al. Transcription factor based therapeutics: drugs of the future?. Trends in Biotechnology. January 1993. Vol. 11. pages 11-18, see entire document.	1-15
A	BURATOWSKI et al. Mechanisms of Gene Activation. Science. 15 December 1995. Vol. 270. pages 1773-1774, see entire document.	1-15
A	POON et al. Immunopurification of Yeast TATA-binding Protein and Associated Factors. The Journal of Biological Chemistry. 25 July 1993. Vol. 268. No. 21. pages 15325-15328, see entire document.	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 MAY 1997

Date of mailing of the international search report

09 JUL 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/06170

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	POON et al. Yeast Taf170 is Encoded by MOT1 and Exists in a TATA Box-binding Protein (TBP)-TBP-associated Factor Complex Distinct from Transcription Factor IID. The Journal of Biological Chemistry. 16 September 1994. Vol. 269. No. 37. pages 23135-23140, see entire document.	1-15
A, P	US 5,534,410 A (TJIAN et al) 09 July 1996, see entire document.	1-15
A, P	US 5,569,588 A (ASHBY et al) 29 October 1996, see entire document.	1-15

Form PCT/ISA/210 (continuation of second sheet)(July 1992)★

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/06170

## A. CLASSIFICATION OF SUBJECT MATTER IPC (6)

C07K 14/40, C12N 1/00, 5/10, 15/31, 15/63; C12P 21/04, 21/06; C12Q 1/02, 1/68; G01N 33/53

## A. CLASSIFICATION OF SUBJECT MATTER: US CL

435/6, 7 8, 29, 69.1, 70 1, 71 1, 243, 320 1, 325; 530/350; 536/23.74

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used)

### APS, DIALOG

search terms: TBP, tata binding protein, tata box binding protein, transcription factor, candida albicans, tfid, screen?, assay?, transcri?, inhibit?, detect?, identity?, compound?, drug?, candidate, pharmaceutic?, rna polymerase n, rna polin, complex?, administer?, treat?, tfidb

